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The Effect of Natural and Anthropogenic Barriers on the Dispersal and Re-establishment Potential of Gambusia holbrooki in Freshwater Systems in the Sutherland Shire

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The Effect of Natural and Anthropogenic Barriers on the Dispersal and Re-establishment Potential of Gambusia holbrooki in Freshwater Systems in the Sutherland Shire

Abstract

For present and future conservation and management programs to be successful in mitigating the spread of the invasive species, *Gambusia holbrooki*, in freshwater systems, it is imperative for us to understand their patterns of dispersal and mechanisms of establishment. Understanding how this species disperses and establishes in a range of freshwater environments will allow for a more thorough understanding of their movement and thus will contribute to more effective management strategies. The present study aims to determine how physical and chemical barriers affect the dispersal potential of *G. holbrooki* in freshwater systems in the Sutherland Shire, NSW, and whether this species has the capacity to re-establish in the short-term in environments they previously inhabited. By using the tag-release-recapture method (Peterson, 1896), visible implant elastomers (VIE) were administered to 700 fish, split into two different populations (red and yellow) that were separated by a trash-rack barrier at each of the two creek sites. Re-capture efforts occurred weekly for 9 weeks, with the movements of the two populations recorded at each site at each location. Re-establishment estimates were determined weekly using presence-absence based observation, by haphazardly netting in areas previously inhabited by *G. holbrooki*. Results of this study highlight that there was no chemical barrier, or natural physical barrier present that hindered *G. holbrooki's* dispersal potential or their ability to re-establish in creeks previously inhabited; even in sites with considerable evidence of petrochemical pollution. However, this study did highlight that anthropogenic barriers such as trash racks have the ability to hamper the dispersal of *G. holbrooki*, providing the rack has at least a moderate amount of trash accumulated. Results also highlighted that *G. holbrooki* populations were apparently unable to re-establish in areas they previously inhabited within the short term. The mosquitofish populations have shown variation in their dispersal pattern depending on site specific influences such as the number and type of barriers, the physico-chemical conditions, landscape structure, and trash rack condition. This provides the first demonstrative link between *G. holbrooki* and the effect of both physical and chemical barriers present in urbanised freshwater systems in the Sutherland Shire. The implication this would have on conservation and management strategies includes devising approaches towards successful mitigation of *G. holbrooki* dispersal in various freshwater systems in NSW.

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The Effect of Natural and Anthropogenic Barriers on the Dispersal and Re-establishment Potential of *Gambusia holbrooki* in Freshwater Systems in the Sutherland Shire

Alicia Mary Cronly

A thesis submitted in part fulfilment of the requirements of the Bachelor of Environmental Science in the School of Earth and Environmental Sciences, Faculty of Science, Medicine and Health, University of Wollongong 2015

October 2015

DECLARATION

The information in this thesis is entirely the result of investigations conducted by the author, unless otherwise acknowledged, and has not been submitted in part, or otherwise, for any other degree or qualification.

Alicia Cronly

20/10/2015

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ABSTRACT

For present and future conservation and management programs to be successful in mitigating the spread of the invasive species, *Gambusia holbrooki*, in freshwater systems, it is imperative for us to understand their patterns of dispersal and mechanisms of establishment. Understanding how this species disperses and establishes in a range of freshwater environments will allow for a more thorough understanding of their movement and thus will contribute to more effective management strategies. The present study aims to determine how physical and chemical barriers affect the dispersal potential of *G. holbrooki* in freshwater systems in the Sutherland Shire, NSW, and whether this species has the capacity to re-establish in the short-term in environments they previously inhabited. By using the tag-release-recapture method **(Peterson, 1896)**, visible implant elastomers (VIE) were administered to 700 fish, split into two different populations (red and yellow) that were separated by a trash-rack barrier at each of the two creek sites. Re-capture efforts occurred weekly for 9 weeks, with the movements of the two populations recorded at each site at each location. Re-establishment estimates were determined weekly using presence-absence based observation, by haphazardly netting in areas previously inhabited by *G. holbrooki*. Results of this study highlight that there was no chemical barrier, or natural physical barrier present that hindered *G. holbrooki*'s dispersal potential or their ability to re-establish in creeks previously inhabited; even in sites with considerable evidence of petrochemical pollution. However, this study did highlight that anthropogenic barriers such as trash racks have the ability to hamper the dispersal of *G. holbrooki*, providing the rack has at least a moderate amount of trash accumulated. Results also highlighted that *G. holbrooki* populations were apparently unable to re-establish in areas they previously inhabited within the short term. The mosquitofish populations have shown variation in their dispersal pattern depending on site specific influences such as the number and type of barriers, the physico-chemical conditions, landscape structure, and trash rack condition. This provides the first demonstrative link between *G. holbrooki* and the effect of both physical and chemical barriers present in urbanised freshwater systems in the Sutherland Shire. The implication this would have on conservation and management strategies includes devising approaches towards successful mitigation of *G. holbrooki* dispersal in various freshwater systems in NSW.

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1. INTRODUCTION

1.1 THE PROCESS OF BIOLOGICAL INVASION

Biological invasion refers to the spread, establishment and persistence of a non-native (or non-indigenous or exotic) species into a habitat that exists beyond the species native range and/or distribution (Garcia-Berthou, 2007; Lockwood *et al*, 2007). These invading species usually challenge the maintenance of biodiversity and natural resources in an ecosystem (Simberloff *et al*, 2013) and have lasting impacts on the biotic and/or abiotic elements of that ecosystem (Moyle and Marchetti, 2006). There are three key stages that are characteristic of the process of non-native species invasion into a new habitat, according to Leprieur *et al*, (2008). These include the initial dispersal of the species; the establishment of self-sustaining populations; and the continual spread into adjacent recipient habitats. The most important stage of this invasion process is arguably the initial dispersal of the species. Without this stage, establishment and spread cannot occur.

The initial dispersal of an invasive species into a non-native habitat is dependent on natural abiotic (physical and chemical) and biotic (community structure and function) attributes of the ecosystem. Anthropogenic factors such as the introduction of biological control agents and human disturbance regimes have played a significant role in global species invasions (Leprieur *et al,* 2008; Moyle and Marchetti, 2006). One of the main factors includes the introduction of exotic species for biological controls. Examples of these include the introduction of the Eastern mosquitofish (*Gambusia holbrooki)* and Cane toad (*Bufo marinus*/*Rhinella marina)* into Australia as biological control agents to aid in the regulation of mosquito populations (NPWS, 2003) and the grey-backed cane beetle, respectively (Markula *et al*, 2010). In order to prevent further non-native invasions in the future, a focus on dispersal control must be at the forefront of research.

1.2 FACTORS THAT PROMOTE INVASION SUCCESS

There have been multiple hypotheses that have suggested possible factors that contribute to successful biological invasion. The leading hypotheses in the field include the “human induced invasion” hypothesis, the “biotic resistance” hypothesis, the “enemy release” hypothesis and the "novel weapons" hypothesis (Callaway and Ridenour, 2004; Colautti *et al*, 2004; Jeschke and Genovesi, 2011; Jeschke *et al*, 2012; Leprieur *et al,* 2008;Liu and Stiling, 2006; Shea and Chesson, 2002). The "human induced invasion" hypothesis suggests that human activity aids in the dispersal and establishment of a species into a new habitat, whether it be directly via biological control, or indirectly via human land use practices. The "biotic resistance" hypothesis suggests that those environments with higher biodiversity are more resistant against invaders due to niche and resource availability being limited. The "enemy release" and "novel weapon" hypotheses suggest that the absence of predators and possession of certain traits will allow successful invasion as a result of having a competitive advantage in that habitat. It is important to note that there is not just one theory that adequately explains all characteristics and factors of biological invasion (**Van Dyke, 2008**). However, these theories are amongst the most supported in the field to date.

For a species to become a successful invader, they must possess certain characteristics and traits that will provide them with a competitive advantage in that specific habitat. But since there are many different types of habitats with a large range of varying abiotic and biotic characteristics, all successful invading species are unlikely to share exactly the same traits and behaviours. However, there are three characteristics that are consistent with all successful invaders (Van Dyke, 2008): (1) Invading species have a high reproductive output and/or multiple breeding episodes each breeding season; (2) invading species are able to survive in unfavourable conditions at low densities until favourable conditions occur, and (3) invading species are able to exploit local conditions that favour the proliferation and persistence of the species and have a wide range of ecological and physiological tolerances. Additionally, the life history characteristics of invading species play a large role in invasion success, with r-selected, opportunist and generalist species being the most successful (Moyle and Marchetti, 2006; Rosecchi *et al,* 2001). Abiotic factors such as space/niche availability, weather regimes and chemical pollutants can have a significant effect on the establishment success of an invading species. Abiotic factors are therefore equally as important as biological factors in certain cases, especially if the invading species has a smaller range of physico-chemical tolerances (Moyle and Light, 1996; Ross *et al,* 2001).

1.3 POTENTIAL IMPACTS OF A SUCCESSFUL INVADER ON ITS ENVIRONMENT

Abiotic Impacts

Invasive organisms are able to directly or indirectly alter the chemistry and physical environment of an ecosystem as a result of their behavioural, reproductive and physiological traits (Mooney and Cleland, 2001). The most prevalent is the direct degradation of the physical habitat such as disturbance to vegetation and sediment (NPWS, 2003). Additionally, alterations to soil and water chemistry can subsequently cause alterations in energy and nutrient flows, as well as the water cycle. This can potentially change the functional processes and physical structure of an ecosystem (Charles and Dukes, 2007). If there is any change in ecosystem processes and functions such as changes in productivity, rate of decomposition and water use efficiency (Figure 1.1), there will also be consequences for the biotic communities that rely on these stable and consistent abiotic conditions and microclimates. Through this type of habitat modification, invasive species have the potential to homogenise habitats at a regional scale and thus eliminate certain microclimates that contribute to the diversity of an ecosystem (Mooney and Cleland, 2001).

Biotic Impacts

The major biological impacts that invasive species have had on ecosystems involve the change in biodiversity and biological community in terms of composition and biological interactions (Charles and Dukes, 2007) (Figure 1.1). Local extinctions and the loss of endemic species are at the forefront of invasive species impact analysis, with uncommon species being considerably more vulnerable when human activity is present (Garcia-Berthou, 2007; Ross, 1991). The main mechanisms that cause this degradation of biodiversity and alteration of biological interactions include niche displacement, competitive exclusion and predation (Mooney and Cleland, 2001). By out-competing other native and endemic species for resources and shelter, native species either have to relocate to another suitable habitat or are forced to seek alternative food and shelter sources. This results in an alteration of the native species' niche, and can contribute to the restructuring of biotic assemblages in that community (Ross, 2001).

Invasive species also have the potential to harbour and transmit diseases and parasites into populations when establishing in new habitats (Henderson, 2009; Ross, 2001). This is potentially dangerous to animal, plant and human populations if there is a large and uncontrollable outbreak of disease or parasitic infection. Introduced and invasive species such as cane toads, rabbits, foxes and European carp have brought in a variety of diseases and parasites from overseas which have also affected the ability of native and endemic species to survive (Henderson, 2009). For example, European Carp are alleged to have introduced the Asian fish tapeworm (*Bothriocephalus acheilognathi*) into Australia, which is also a threat to native fish species such as the Murray cod, golden perch and silver perch (Henderson, 2009).

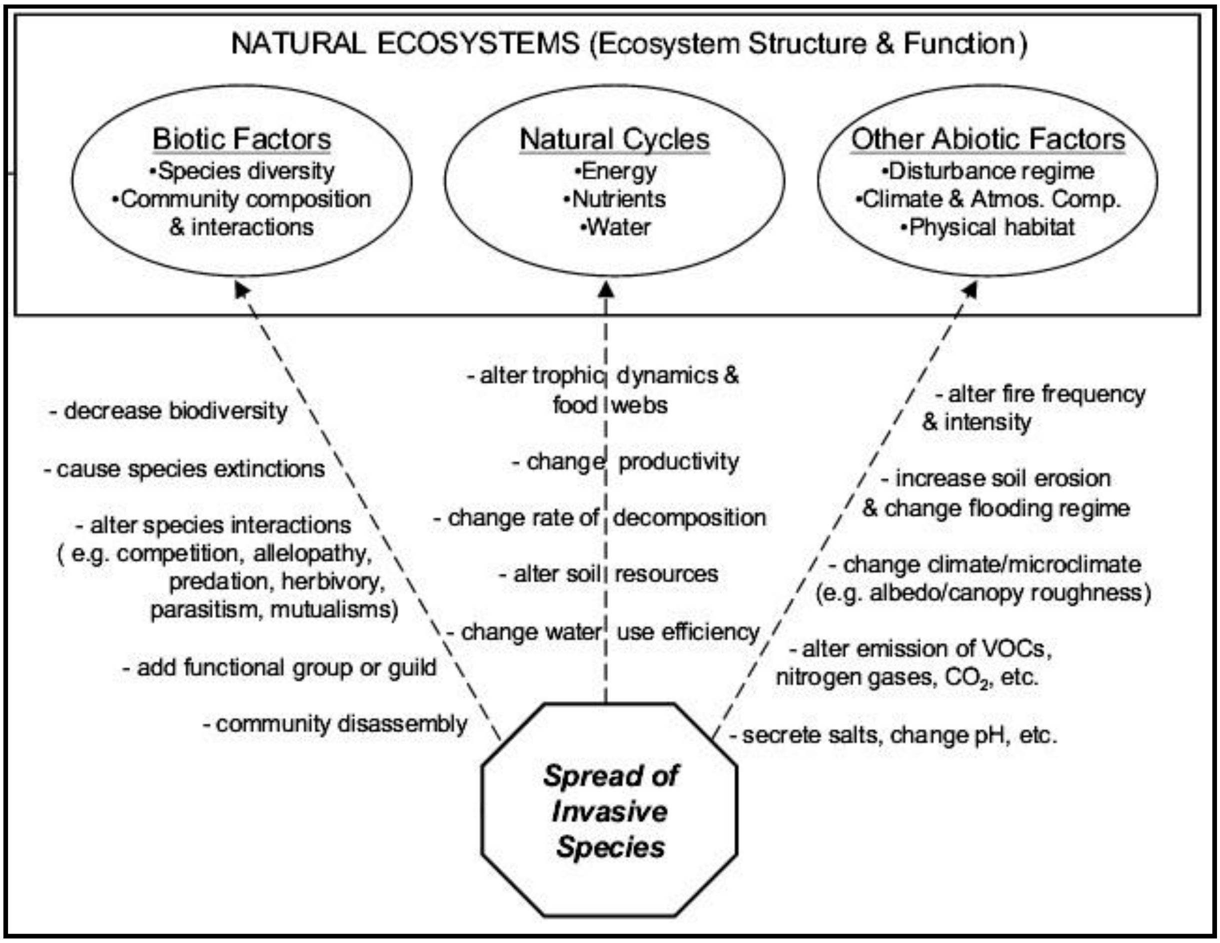

Figure 1.1 Impacts of Invasive Species on Biotic and Abiotic Factors, and Natural Cycles. Image taken from Charles and Dukes, (2007).

1.4 HISTORY OF GAMBUSIA HOLBROOKI INTRODUCTION

1.4.1 SPECIES ORIGIN AND ENTRY INTO AUSTRALIA

Gambusia holbrooki are native to the coastal drainages of south-eastern USA and parts of North-eastern Mexico (Clarke *et al,* 2000). In the USA, their distribution extends from southern Mississippi and Alabama, to Florida in the south, and up the eastern coastline towards New Jersey (Wooten *et al,* 1988) (Figure 1.2).

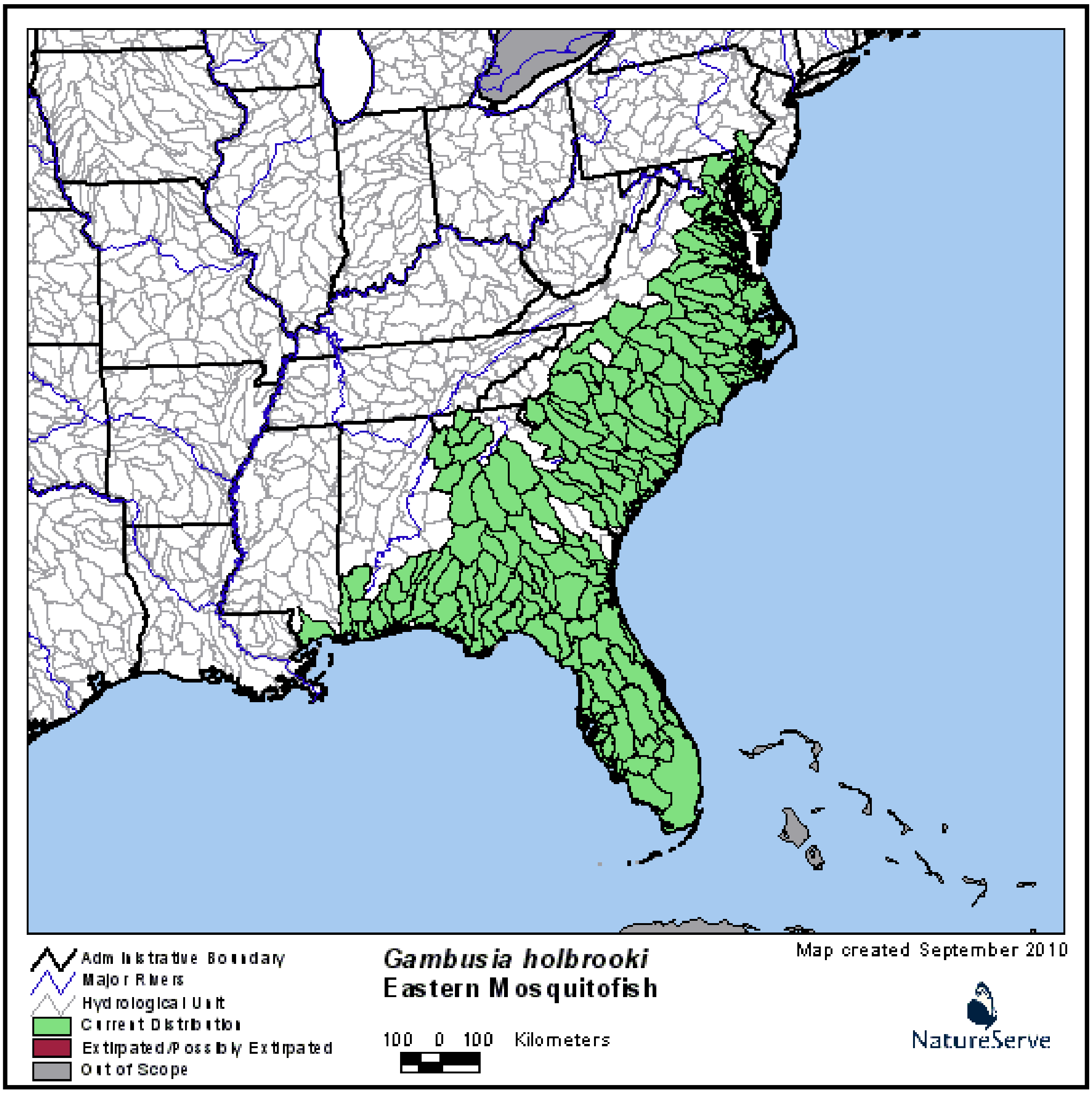

Figure 1.2 Current distribution of* Gambusia holbrooki *in south-eastern U.S.A. Image taken from Nature Serve website (2015): [http://explorer.natureserve.org/servlet /NatureServe?searchName=Gambusia+holbrooki].

Gambusia holbrooki were first introduced into Australia in 1925 from Georgia in the U.S.A. (Wilson, 1960). They were introduced as a biological control agent in an attempt to reduce mosquito populations, and were first released into the Sydney Botanical Gardens on the approval of the Chief Health Inspector of the City of Sydney (McKay, 1984). From 1927 onwards *G. holbrooki* were widely distributed throughout the state of NSW, and in 1940 they were flown interstate to Darwin in the Northern Territory and were spread through freshwater environments in and near military camps at the time (Boulton and Brock, 1999). It was not until the late 1960's that *G. holbrooki* were distributed by humans in the Illawarra and Central Coast regions (NPWS, 2003). It was only in 1982 that the World Health Organisation (WHO) announced that they no longer recommended using *G. holbrooki* as a biological agent to reduce mosquito numbers, due to the consequential impacts it has had on native fauna (Legner, 1996).

1.4.2 GAMBUSIA HOLBROOKI AS A BIOLOGICAL CONTROL AGENT

During the early 1900's when it was realised that malaria and yellow-fever were transmitted to humans via mosquitoes, there was considerable interest in finding a simple and effective solution to drastically reduce mosquito populations at the larval stage (Boulton and Brock, 1999). Along with physical and chemical control methods, natural control methods were also researched, which led to the concept of biological control (Lloyd, 1990). The first study of *Gambusia holbrooki* as a biological control agent for mosquito larvae was conducted in Texas in 1905 (Wilson, 1960). Soon after, *G. holbrooki* became known as the best biological control agent for mosquitoes on a global scale due to the capacity for the species to tolerate a wide range of environmental variables.

In Australia it was initially thought that *G. holbrooki* was quite effective at managing mosquito larvae populations in permanent pond habitats (Wilson, 1960). However as more introductions took place around the state, it was found that "...their effect on mosquitoes [was] negligible..." (Grant, 1978). Additionally, it was found that *G. holbrooki* had a significant negative ecological impact on native amphibians and fish, which greatly outweighed their benefit as a control agent, and thus the use of them as a mosquito control agent was discontinued (Legner, 1996). Further gut analysis studies on *G. holbrooki* in Australia also indicated that mosquito larvae only make up a very small proportion of their diet, supporting the notion that they are not an effective mosquito predator (Lloyd, 1984).

1.4.3 DISTRIBUTION OF GAMBUSIA HOLBROOKI

In Australia, *Gambusia holbrooki* are widely distributed and found in all states and territories (OZCAM, 2015)(Figure 3). *Gambusia holbrooki* populations have had the most success establishing in south-eastern Australia, and are a very common species throughout New South Wales, South Australia, Queensland and Victoria in both inland and coastal drainages (NPWS, 2003 and Pyke, 2008). Tasmania remained free from established *Gambusia* populations until 1992, where a population was found in a farm dam in the Tamar River catchment (IFS, 2014; Lynch, 2008). The efforts of the Inland Fisheries Service (IFS) proved effective until November 2000, when a population of over 1500 individuals was recorded to have re-established in the Upper Tamar Estuary (Keane and Neira, 2004). Since then, published records of *G. holbrooki* in Tasmania have been limited.

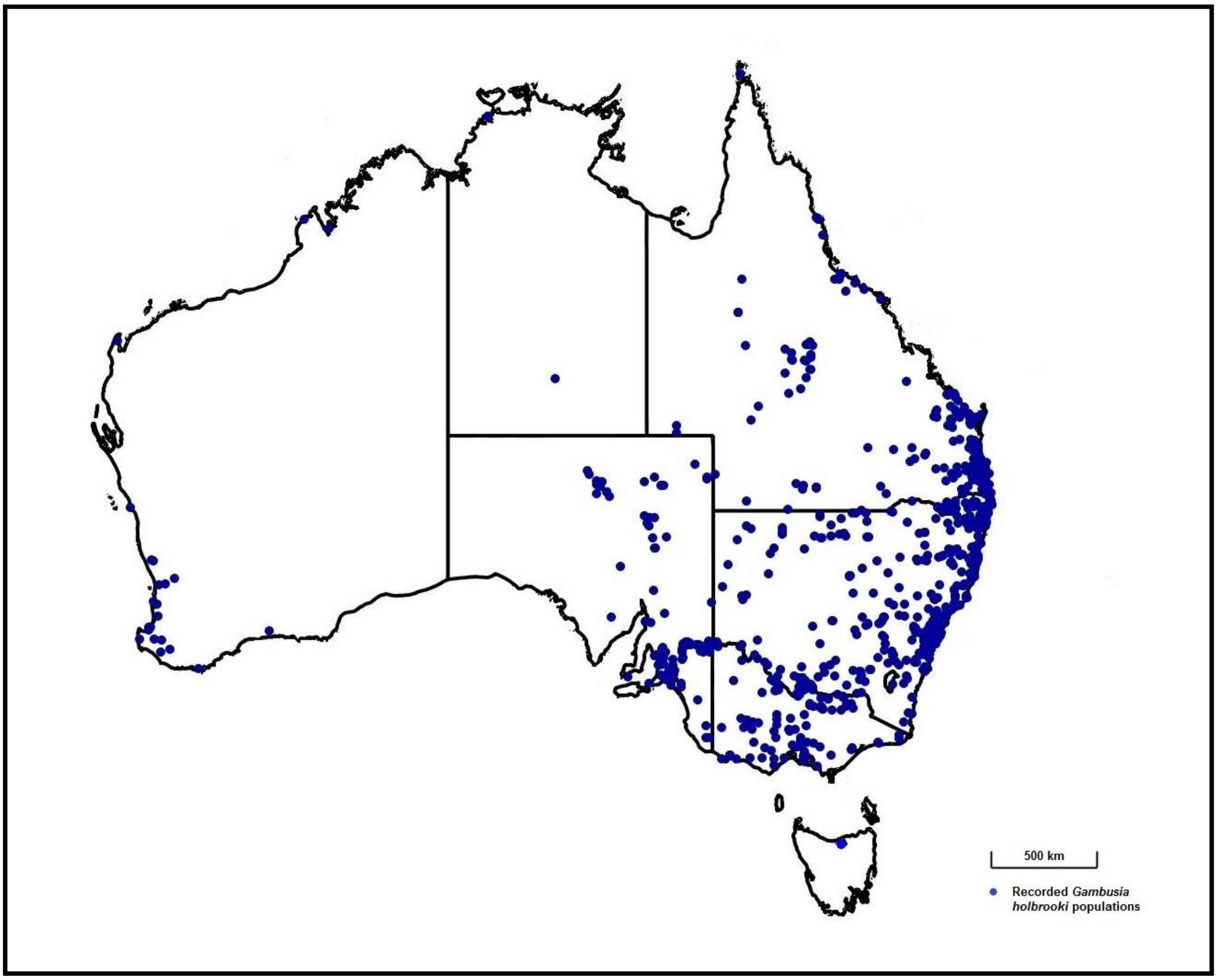

***Figure 1.3. Distribution of* Gambusia holbrooki *in Australia. Image taken from the Online Zoological Collections of Australian Museums (OZCAM) website (2015): http://ozcam.ala.org.au/occurrences/search?q=gambusia+holbrooki&qc=datahubuild:dh1&fq=species:%22Gambusia+holbrooki%22#tabmapView*.**

In New South Wales specifically, *Gambusia holbrooki* are prevalent in the Murray-Darling River system, Sydney Basin, Illawarra catchment and numerous areas in Lake Macquarie in the Central Coast (Goldingay and Lewis, 1999; NPWS, 2003). In particular, populations are most common and widespread along the NSW and Queensland coastline. Limited surveys of *G. holbrooki* distributions have been conducted in recent times; however the most comprehensive to date is the NSW Rivers Survey, published in 1997. This identified populations of *G. holbrooki* at 27 of the 80 sites surveyed in the state (Harris and Gehrke, 1997)(See Appendix 1). It is evident that this number has grown since then, with a significant rise in the recorded occurrences of *G. holbrooki* populations from 2000-2010 (OZCAMa, 2015).

On a global scale, *Gambusia holbrooki* and sister species *Gambusia affinis* (western mosquitofish) are now established in over 67 countries (Garcia-Berthou *et al,* 2005), with the genus present on all continents except Antarctica (Courtenay and Meffe, 1989). The wide, global spread of *Gambusia* was the direct result of anthropogenic introductions during the early to mid 1900's; with only a small fraction being native to the region or able to naturally disperse significant distance (NPWS, 2003). The ability of this genus to tolerate a wide range of ecological conditions in the absence of natural predators is the primary reason why *Gambusia* has continued to persist on a global scale (Webb *et al,* 2007).

1.5 STUDY AIMS AND QUESTIONS

Using *Gambusia holbrooki* as a model species, this study aims to identify the dispersal patterns and re-establishment potential of *G. holbrooki* in freshwater creek systems in the Sutherland Shire. In particular, this study aims to answer the following:

1. How far are *G. holbrooki* able to disperse in freshwater creek systems over a given time period?
2. Are *G. holbrooki* populations able to re-establish in freshwater creeks that they previously inhabited?
3. Do physico-chemical, environmental and anthropogenic factors influence dispersal and re-establishment?

2. LITERATURE REVIEW

2.1 AIMS AND BACKGROUND

The main aim of this literature review is to understand the ecological and physical factors that can potentially influence the dispersal potential and re-establishment of *Gambusia holbrooki* in an aquatic system. It will also focus on the interactions between *G. holbrooki* and the biotic and abiotic environment, and how this influences their abundance and tendency to disperse. This review defines what is already known and understood about *G. holbrooki* and identifies important knowledge gaps and limitations. In particular, this review is beneficial for establishing knowledge about their ecological preferences, which species they compete with, the potential impacts *G. holbrooki* will have on their environment, and barriers that could help to control already established populations. These parameters are all important in recognising why and how invasive pests disperse and establish as they do. Finally, this review aims to determine the most efficient and precise methodology to quantify how far they can potentially disperse in a freshwater system over a given time frame.

2.2 BIOLOGY AND ECOLOGY OF GAMBUSIA

2.2.1 BIOLOGY AND SOCIAL ORGANISATION

Gambusia holbrooki populations can grow rapidly in optimal conditions. Females have a larger size range than males, reaching up to 6cm and males 3.5cm (McGrouther, 2012). The sexual maturity of females can occur between 18-28 days, with males reaching sexual maturity a bit later from 43-62 days (Meffe, 1992). Females can produce multiple broods within a single season ranging from 2-6 broods (Meffe, 1992; McDowall, 1996) and brood sizes can range from 10-100 live young (Brown-Peterson and Peterson, 1990). The variation in the number of broods per season and their sizes is dependent on water temperature, age and nutrient availability (Mulvey *et al,* 1994). As female *G. holbrooki* are viviparous organisms, the mortality rate of their young is lower compared to oviparous fish, whose eggs can be easily destroyed or preyed upon. As a result, population size can grow at a very fast rate (Spencer *et al*, 2000). The mean abundance of *G. holbrooki* populations is at the highest in the summer months, as a result of prolific breeding around mid-November each year (Lynch, 2008). This breeding is regulated by photoperiod, with the breeding season ending when day length falls below 13hrs, even when all other variables are favourable (Lloyd *et al*, 1986).

Group behaviour studies on *Gambusia holbrooki* have demonstrated that social facilitation is an important concept in the behavioural responses of this species. In larger social groups, *G. holbrooki* have a tendency towards increased exploration of the environment, whereas in smaller groups, this behaviour is lessened due to a perceived increase in risk (Ward, 2012). This retreat in exploratory behaviour is also evident when chemical cues pertaining to an injured conspecific are present (Martin, 1975; Ward, 2012).

The rate of exploration in their environment can therefore influence their dispersal potential in a freshwater body. A behavioural impact study of *G. holbrooki* on the native Pacific Blue-Eye (*Pseudomugil signifier*) only demonstrates aggressive behaviour by male *G. holbrooki* towards *P. signifer* males that are perceived by *G. holbrooki* as competition for copulation (Howe, 1995). However, in terms of competition for shelter and food, *G. holbrooki* did not show any evidence of territoriality (Howe, 1995). A study by Toft *et al* (2004) also discovered that altered social and reproductive behaviours can occur when androgenic or hormone-altering chemicals in effluent streams reach *G. holbrooki* populations and alter their natural behaviours. This potentially has relevance to many *G. holbrooki* populations in urban creeks and water bodies near industrial estates where pollution is more prominent.

2.2.2 HABITAT PREFERENCES

Gambusia holbrooki have shown a preference for freshwater bodies that are shallow, warm with at least a moderate amount of aquatic vegetation (Lloyd *et al*, 1986; Lund, 1999 and Webb *et al*, 2007). They are almost always found in freshwater habitats such as drains, creeks, rivers, dams and streams that have low discharge and flow rates, and submerged vegetation cover (Cadwallader and Backhouse, 1983; Meffe and Snelson, 1989). Aquatic habitats with these characteristics enhance *G. holbrooki*'s survivability (Lloyd *et al*, 1986). Systems with high disturbance and naturally variable discharge regimes are unfavourable for sustaining *G. holbrooki* populations and can almost eliminate populations due to their inability to tolerate these harsh, physical conditions (Galat and Robertson, 1992).

Within these warm, still, shallow and highly vegetated habitats, *G. holbrooki* are very flexible in terms of chemical environmental tolerances. They can survive in water temperatures ranging from 0.5°C to 44°C and are reproductively active between 18°C and 38°C, but prefer temperatures between 25°C and 33°C (Webb *et al*, 2007). They can withstand a pH ranging from 4.46 to 10.2 based on both field and lab observations (Swanson *et al,* 1996; Luna, 2001), and can tolerate salinity levels from 0-41 ppt (Hubbs, 2000). They are able to survive in conditions where the dissolved oxygen is as low as 0.2 – 11 mg/L, (the former far below the limits of most Australian fish) (Lloyd, 1990), and can occupy both turbid and non-turbid environments from 3-275 Jackson Turbidity Units (Cherry *et al*, 1976).

2.2.3 DISPERSAL MECHANISMS

Invasive *Gambusia holbrooki* exhibit a greater dispersal tendency than other Poeciliidae fish, being able to disperse as juveniles and travel farther than their fish relatives (Rehage and Sih, 2004). This dispersal behaviour has been suggested to be influenced by *G. holbrooki's* 'boldness', which has been defined as the "propensity of organisms to move through and explore an unfamiliar place" (Fraser *et al*, 2001 and Wilson *et al*, 1993). Recent research by Cote *et al* (2010) suggests that a species 'personality trait' (i.e. boldness, sociability, activity and exploration tendency) is an important indicator of dispersal distance in *G. holbrooki*. This underlying behavioural trait could influence the variation in dispersal abilities between *G. holbrooki* and other native and non-native aquatic fish. This explains, at least partly, why *G. holbrooki* is such a successful coloniser. However, studies of this in a non-artificial environment for *G. holbrooki* have yet to be conducted.

The major non-behavioural mechanisms that have seen *Gambusia holbrooki* continue to colonise and establish in NSW include: dispersal by humans (both direct and indirect); naturally via floods, stormwater and irrigation channels; and by other animals such as migratory and wetland birds (Chapman and Warburton, 2006; Lintemans, 2004; McNeely, 2001; NPWS, 2003). Initially, human dispersal of *G. holbrooki* in NSW was encouraged as they were used as a biological control agent for mosquito populations. Presently, humans are helping to disperse *G. holbrooki* by unintentionally or intentionally releasing them without knowing their negative biological effects (McNeely, 2001; NPWS, 2003 and Pyke, 2008). The misidentification of *G. holbrooki* as a native species is contributing to the continual dispersal of *G. holbrooki* (Lintermans, 2004 and NPWS, 2003).

The post-flood movement of *G. holbrooki* and the effects of large rain and storm events have, in some cases, allowed *G. holbrooki* to disperse and migrate through storm-water drainage systems and irrigation channels (Chapman and Warburton, 2006). This has allowed them access to environments that are out of their natural range of migration, and thus given them the ability to colonise freshwater streams and creeks that are naturally disconnected. It has been suggested that juvenile *G. holbrooki* can be dispersed by wetland birds by transporting them during flight on their plumage or feet (NPWS, 2003). Studies observing how natural and anthropogenic barriers influence *G. holbrooki* have not yet been analysed in much detail. By implementing more field experimentation into such studies we can potentially determine site specific parameters that may help in understanding how this species is able to migrate and infiltrate into other aquatic systems.

2.2.4 KNOWN AND POTENTIAL DISEASES, COMPETITORS AND PREDATORS

The successful establishment of *Gambusia holbrooki* has been correlated with their tolerance towards a large range of environmental and chemical conditions, paired with an efficient reproductive strategy that yields low mortality of young (NPWS, 2003; Pyke, 2008). To be able to naturally and effectively regulate invasive *G. holbrooki* populations, there needs to be a presence of predators and competitors in their environment to regulate and control abundance. There are limited studies suggesting the presence of *G. holbrooki* predators in Australia; however in the U.S. large predatory fish, wading birds, invertebrates and snakes are natural predators of *G. holbrooki* (Meffe and Snelson, 1989). Potential predators in Australia could also be birds, fish and even spiders, with some reports suggesting birds such as little black cormorants (*Phalacrocorax sulcirostris*) are able to feed on exotic fish such as carp and *G. holbrooki* in NSW inland lakes (Boulton and Brock, 1999). Suggestions as to why aquatic predators have a low or negligible predatory impact on *G. holbrooki* is that the fish are unpalatable compared to other food sources, or native predatory species have not adapted strategies or behaviours to effectively capture *G. holbrooki* (Lloyd, 1984).

Competition exists between *Gambusia holbrooki* and any other aquatic organism where there is overlap in their dietary requirements and preferred habitat. This has been evident between *G. holbrooki* and species such as the native Pacific Blue-Eye and tadpole species which also share the same type of vegetated, shallow habitats. In NSW, only one known parasitic protozoan (*Goussia piekarskii*) has been found to have adverse effects on the survivorship of *G. holbrooki* (Lom and Dyková, 1995). The presence of predators, competitors and parasites will help to regulate *G. holbrooki* abundance, and could potentially decrease their invasion success in environments where these threats are present.

2.3 IMPACTS OF GAMBUSIA

2.3.1 IMPACTS ON VEGETATION AND RIVER HEALTH

There has been no significant evidence that *Gambusia holbrooki* directly disturbs and disrupts the physical habitat of an aquatic environment. However, large populations of *G. holbrooki* can adversely affect water quality by reducing zooplankton communities via predation and consequently trigger phytoplanktonic blooms (Hurlbert *et al*, 1972; Kennard *et al*, 2005). The persistence of stable and diverse zooplankton assemblages helps to regulate levels of phytoplankton in rivers and streams (Rowe, 2008). Additionally, *G. holbrooki* do not show any obvious behaviours that may contribute to the degradation of the aquatic environment such as disturbance of sediment, or damage to aquatic vegetation (NPWS, 2003). In high abundances, *G. holbrooki* can cause an increase in nitrogen and phosphorous from excretion and thus alter the chemistry of freshwater bodies (Akhurst *et al*, 2012). This is especially detrimental to smaller creeks and ponds that cannot dilute or regulate this increase in nutrient load, contributing further to the establishment of algal blooms (Hurlbert *et al*, 1972). Additional research of *G. holbrooki* in various natural systems is needed to be able to tease apart the importance of these broader environmental effects versus the more localised biological interactions.

2.3.2 BIOTIC IMPACTS

Impacts on Invertebrates

Impact studies on *Gambusia holbrooki* have been largely focused on how aquatic macro-invertebrate assemblages are altered as a result of *G. holbrooki's* predatory behaviour in a freshwater environment (Lloyd, 1984; Lloyd *et al*, 1986; Lloyd, 1990 and Margaritora *et al*, 2001). Many studies have highlighted a decrease in abundance and richness of macro-invertebrate taxa such as rotifers, cladocerans, copepods, ephemeroptera and anisoptera larvae in the presence of well-established *G. holbrooki* populations (Anstis, 2002; Hurlbert et al, 1972; Lloyd, 1984; Lawler *et al*, 1999 and Pope and Hannelly, 2013). The implications of altered macro-invertebrate taxa include consequential changes to water quality, and threats to species that rely on macro-invertebrates as a primary food source such as tadpoles (Lloyd, 1990; Lund, 1999 and Margaritora *et al*, 2001). From this research it has been suggested that in relatively pristine environments, *G. holbrooki* in high densities has the potential to locally eradicate rare species and may actually encourage mosquito larvae by eating their invertebrate predators in preference to the mosquito larvae (Lloyd et al, 1986; Lund, 1999).

Impacts on Amphibians

Research into the impacts of *Gambusia holbrooki* on amphibians increased in the 1990's when it was evident that amphibian numbers in south-eastern Australia started to decline rapidly (Pyke, 2008). Australian studies have highlighted declines in frog species directly caused by *G. holbrooki* (Hamer and Parris, 2013 and Hunter *et al*, 2011) including: *Litoria aurea* (Green and Golden Bell Frog)*, Litoria dentata* (Bleating Tree Frog), *Litoria booroolongensis* (Booroolong Frog), *Limnodynastes peronnii* (Striped marsh frog), *Crinia signifera* (Common Eastern Froglet) and *Geocrinia victoriana* (Eastern Smooth frog) (Hamer *et al*, 2002; Hamer and Parris, 2013; Hunter *et al*, 2011; Morgan and Buttemer, 1996; Pyke and White, 2000 and Webb and Joss, 1997). *Gambusia holbrooki* directly impacts the decline of these species by preying upon their tadpoles and eggs as well as inflicting injury upon larger tadpoles, such as tail nipping (NPWS, 2003; Pyke and White, 2000; Pyke, 2008 and Reynolds, 2009). Some tadpole species are shown to be more vulnerable to predation than others through differences in predator avoidance mechanisms and behaviours (Hamer *et al*, 2002 and Morgan and Buttemer, 1996). These behaviours strongly influence their survivorship against *G. holbrooki* and thus some populations are more vulnerable to decline than others (Hamer *et al*, 2002).

Gambusia holbrooki predation on tadpoles and eggs has shown to decrease with increasing vegetation density and habitat complexity, where tadpole mortality by *G. holbrooki* is shown to be highly influenced by a lack of habitat structure and predator avoidance mechanisms (Hamer *et al* 2002; Morgan and Buttermer, 1996; Reynolds, 2009 and Webb and Joss, 1997). However, Morgan and Buttemer (1996) did not account for interactions with other predatory fish that could also be contributing to tadpole decline in their field experimentation. Similarly, Webb and Joss (1997) based their data on direct sampling from adult frog populations. Given that the tadpole life stage is the life stage most at risk from *G. holbrooki,* it seems that sampling tadpoles, not frogs, is the more appropriate approach.

Impacts on Native Fishes

There has been considerable interest in the influence of *Gambusia holbrooki* populations on small native fish species that occupy similar ecological niches. Through direct competition for resources, shelter and food, some native fish may become spatially displaced, and in a given environment, their abundance, body condition and ecological range may be compromised (Galat and Robertson, 1992; Ivantsoff and Aarn, 1999; Lloyd, 1990; Macdonald *et* al, 2008; Macdonald *et al*, 2012). It is through the ecological overlapping of dietary and spatial requirements with *G. holbrooki* that have seen the decline in native Australian fish species such as: *Pseudomugil signifier* (Pacific blue eye), *Nannoperca oxleyana* (Oxleyan pygmy perch), *Craterocephalus fluviatilis* (Murray Hardyhead), *Bidyanus bidyanus* (Silver Perch), *Ambassis agassizii* (Olive Perchlet) and *Mogurnda adspersa* (Southern Purple Spotted Gudgeon) (NSW Fisheries Scientific Committee, 2015; Howe *et al*, 1997). Predation and physical injury by *G. holbrooki* can force these native fish into habitats where exposure to predators is increased or food sources are very limited (Ayala *et al*, 2007).

The direction and extent of *Gambusia holbrooki's* impact on natives appears to also be regulated and dependent on environmental forces and behavioural adaptations of native fish in response to predation and aggression of *G. holbrooki*. Recent research by MacDonald *et al* (2012) suggests that the life history of each species is a major determinant in assessing the extent of impact inflicted upon Australian natives by *G. holbrooki*. Generalist life history strategists are more likely to be relieved from a major proportion of competitive and predatory pressures, allowing a degree of co-existence in a resource limited environment. Conversely, specialist fish species that share the same ecological niche as *G. holbrooki* are more likely to become less resistant against *G. holbrooki* if unable to gain a competitive advantage due to having a narrower ecological niche. Specialist species are more vulnerable to local extinction if they do not exhibit any defensive behaviour that enhances their survivability in an environment where *G. holbrooki* are present (MacDonald *et al*, 2012).

In analysing the existing evidence of the impact of *Gambusia holbrooki* on Australian native fishes, Rowe *et al* (2008) contributes an extensive review and identifies 23 native Australian fish that have been negatively affected by the presence of *G. holbrooki*. The consequential impacts from the reviewed studies included negative effects on: abundance, ecological range, body condition, mortality rates, population fragmentation and reductions in growth and fecundity. These impacts are the direct result of biological mechanisms that *G. holbrooki* implement such as: aggressive interactions, predation (both direct and indirect) and competitive exclusion. However, many studies are based solely on either field experiments (11 of 23) or lab experiments (4 of 23), with only 8 species of native fish negatively affected in *both* lab and field experiments. Conducting both field and lab experimental approaches ensures that both abiotic and biotic factors in an environment are comprehensively analysed to give the most accurate account of what is happening in reality (Ling, 2004). Further insight and research into the biological interactions between *G. holbrooki* and other predatory non-native fish in Australia is recommended to further enhance knowledge of how *G. holbrooki* interact with their environment. The large focus on impact assessments and studies that aim to determine effective control solutions, also rarely look into dispersal mechanisms or re-establishment estimates.

2.4 <u>SAMPLING TECHNIQUES FOR STREAM FISHES</u>

2.4.1 DETERMINATION OF DISPERSAL AND ABUNDANCE

This study focuses on monitoring and analysing how *Gambusia holbrooki* move throughout creek and stream systems in the Sutherland Shire. To be able to monitor these targeted populations we must be able to distinguish between those that are being monitored versus those that are newly recruited populations. When determining the most appropriate method to measure dispersal and abundance in aquatic systems; the complexity of the aquatic system, the ease with which sampling can be standardised, how common the study species is, and the geographic boundary of your study individuals need to be identified. (Pope *et al,* 2010). When selecting a sampling methodology, it is important to consider the amount of bias associated with the sampling technique and the ability to reproduce the technique with precision (Pope *et al,* 2010). This is best achieved by using a systematic sampling design that is able to be replicated with consistency and minimal error. Systematic error can be minimised by using the appropriate equipment for sampling and following a comprehensive design procedure.

Given this, one of the most prevalent sampling methodologies that monitor the dispersal of stream fishes is the capture-mark-release-recapture technique (Thompson, 2003). This technique allows the population of study fish to be distinguished between other individuals within the population, and therefore allows efficient detection of the individuals and their dispersal potential. However for this to be an effective technique, enough individuals need to be tagged so that the chances of re-capture are high. We must also know the geographic boundaries of the aquatic environment in which they live, so that tagged individuals are not lost from the study by means other than perishing. Abundance can also be estimated from this method by using abundance indices such as the 'catch per unit effort' (C/f) estimate (Pope, *et al,* 2010). This gives an approximate value of the population numbers in select streams and creeks and is simple and easy to conduct. Additionally, this method can be used to directly compare relative population size with other study streams that use the same abundance index.

2.4.2 DETERMINATION OF RE-ESTABLISHMENT

Re-establishment refers to the presence of a stable population in a select habitat or region that was once eliminated as a result of an extreme displacement event. This can be the result of an extreme weather event, seasonal cycles, pollution event or exposure to a predator or disease in the population which eventually leads to the population's demise (Pyke, 2008). The ability of an aquatic species to re-establish in the same or a similar habitat depends on the species dispersal mechanisms, habitat fragmentation and connectivity, as well as presence or absence of natural or manmade physical barriers (NPWS, 2003; Rahel, 2013).

In regards to *Gambusia holbrooki,* extreme storm and flooding events have the capability to eliminate stream and creek populations, and are the most common cause of local extinctions in *G. holbrooki* populations (Chapman and Warburton, 2006). By sampling in areas that provide potentially suitable habitats within the creek or stream system, it increases efficiency and probability for locating re-established individuals (Peterson and

Dunham, 2003). Using categorical presence/absence data allows an estimation of how long it takes a species to re-establish in the same environment, if it is able to re-establish at all.

2.5 PHYSICAL AND CHEMICAL BARRIERS AS CONTROL AGENTS FOR GAMBUSIA

Efforts to aid in the control of *Gambusia holbrooki* will not be effective unless we are able to understand how they disperse between aquatic systems, what habitats and environments they prefer and can inhabit, and whether they are able to be outcompeted or preyed upon in these preferred environments. By breaking down the communication and reproductive cues and signals of pest species, or using barriers which interfere with these parameters, then population control can become effective (NSW DPI, 2015).

Physical barriers can fragment fish populations and inhibit connectivity between populations (Rahel, 2013). These barriers can be natural barriers such as fallen trees, a build-up of debris, flood regimes or anthropogenic barriers such as trash racks, weirs, culverts and mesh barriers (Bush Heritage Australia, 2011; NSW DPI, 2015; Rahel, 2013). These barriers can act as effective control agents as they still permit hydraulic connectivity whilst hindering biological connectivity (Rahel, 2013). Physico-chemical control agents can also be natural or anthropogenic such as salinity and pH measures, or synthetic chemical agents such as pesticides and petrochemical pollutants (Kerezsy, 2009). Therefore, assessing both natural physico-chemical barriers and anthropogenic barriers is vital for identifying the patterns of movement and dispersal of *G. holbrooki.*

2.6 CONCLUSION: JUSTIFICATION AND SIGNIFICANCE OF RESEARCH

From this research, much more can be learned about the way invasive species are able to establish and thrive in ecosystems that are already abundant with well adapted native species. The potential implications of this pest not becoming manageable for the Sutherland Shire are first and foremost the disruption of natural biological processes of the aquatic communities in the Royal National Park, which may affect the functionality of these eco-systems and more so, have a lasting impact on aquatic diversity of both vertebrates and invertebrates. To be able to sustain such a rich diversity of functioning ecosystems is to firstly rid it of anything that compromises it. For a species such as *G. holbrooki* that has established on a broader scale along the east coast of NSW and QLD, this research can also benefit other regional areas whose biological diversity and habitat structure are becoming compromised and threatened by this fish species. Not only will this research therefore benefit the Sutherland Shire, but can also be used to benefit the wider communities that are struggling with keeping this pest under control and understanding its migration patterns and mechanisms.

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

3.1.1 REGIONAL SETTING AND SITE SELECTION

All study locations were selected within the Sutherland Shire region, which is located 26km south of the Sydney CBD and covers approximately 370 square kilometres (Sutherland Shire Council, 2015). This region encapsulates a wide diversity of aquatic systems including rivers, streams, creeks, tributaries, wetlands, estuaries, bays and beaches. The Port Hacking River and Georges River are the two major river systems in the region that feed water into these other aquatic environments. Residential land use and bushland dominates the region, however there is a significant representation of commercial and industrial zones as well (Sutherland Shire Council, 2015).

Study locations were selected based on anecdotal evidence of the presence of established *Gambusia holbrooki* populations, since no previous studies of *G. holbrooki* have been conducted in the region. Sites were also selected that have well defined geographic boundaries so that monitored populations and individuals would not be lost from the study by means other than mortality and predation. Two locations were selected from these criteria to monitor the migration and dispersal potential of *G. holbrooki* in an aquatic system using the capture-tag-release-recapture methodology. These locations were Forbes Creek in Engadine (34º03’0.29”S; 151º00’53.4”E) and Marton Park Wetland in Kurnell (34º00’42.7”S; 151°12’53.9”E) (Plate 3.1). Two locations were also selected to determine the rate of re-establishment of *G. holbrooki* in sites which they had inhabited previous to an extreme storm event in April 2015. These locations are Carina Creek in Como (34º00’36.9”S; 151º04’05.6”E) and Gwawley Creek in Sylvania (34º01’14.6”S; 151° 06’04.8”E) (Plate 3.1).

Forbes Creek is part of the Woronora catchment and is located in North Engadine. It extends off the Woronora River system and has connectivity with Loftus Creek downstream. It is an urban creek within a large residential zone (41%) with the remaining land space occupied by bushland (30%), roads (13%) and other hard surfaces (34%) (Sutherland Shire Council, 2015a). It is also equipped with a trash rack located off Lochiel Road, adjacent to Council’s Bushcare site (Figure 3.1, Plate 3.2).

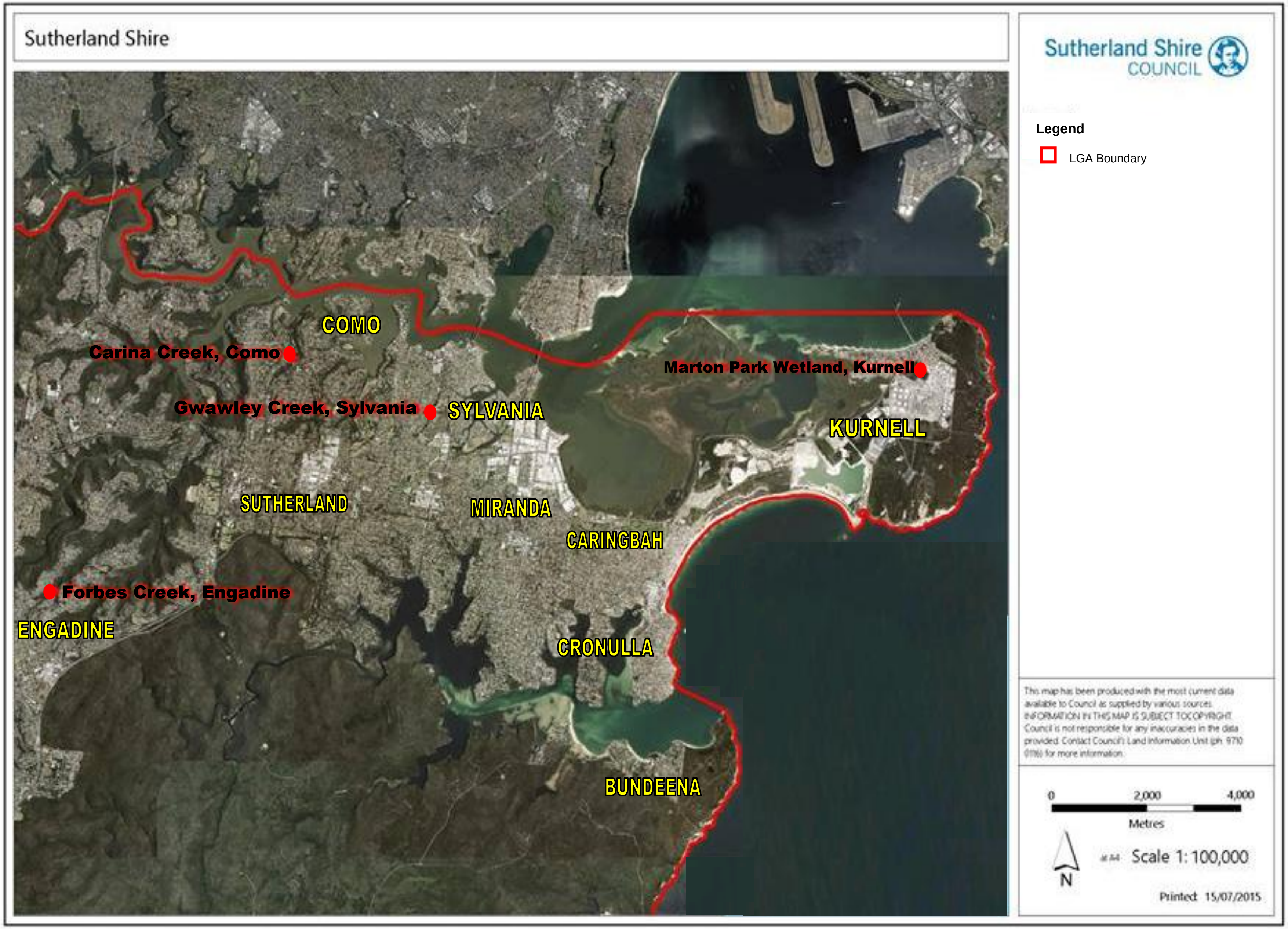

Plate 3.1 Study locations in the Sutherland Shire: Forbes Creek, Engadine; Marton Park Wetland, Kurnell; Carina Creek, Como and Gwawley Creek, Sylvania.

Figure 3.1 Forbes Creek trash rack, Lochiel Road.

Marton Park Wetland is an example of a Sydney freshwater wetland and is located on the Kurnell peninsula in southern shores of Botany Bay. It is a recognised as an endangered ecological community (EEC), and is protected under the NSW Threatened Species Conservation Act for the Green and Golden Bell frog (Sutherland Shire Council, 2009). It is a mixture of industrial, commercial and residential land uses, and is part owned by Sutherland Shire Council, NSW Department of Planning and Caltex Refineries NSW Pty Ltd (Sutherland Shire Council, 2009). It is also equipped with two trash racks at the north-eastern end of the wetland on Cook Street (Figure 3.2, Plate 3.3).

Figure 3.2 Marton Park Wetland trash racks, Cook Street.

Carina Creek is a part of the Georges River catchment and is located in Como. It connects with Carina Bay which is an extension of the Georges River. It is classified as an urban creek with a large residential zone (60%) with the remaining land space occupied by roads (22%) and other hard surfaces (49%) such as the adjacent Carina Bay Reserve (Sutherland shire

Council, 2015b). It is also equipped with a large trash rack adjacent to Honeysuckle Reserve along Wattle Road (Figure 3.3, Plate 3.5).

Figure 3.3 Carina Creek trash rack, Wattle Road.

Gwawley Creek is a part of the Georges River catchment and is located in Sylvania. It flows out to Gwawley Bay in Sylvania Waters and has no other connecting creeks, streams or tributaries. It is classified as an urban creek which is dominated by a large residential zone (62%), with roads (24%) and other hard surfaces (14%) occupying the remaining space (Sutherland Shire Council, 2015c). This creek is also equipped with a large trash rack where Box Road intercepts the creek (Figure 3.4, Plate 3.4).

Figure 3.4 Gwawley Creek trash rack, Box Road.

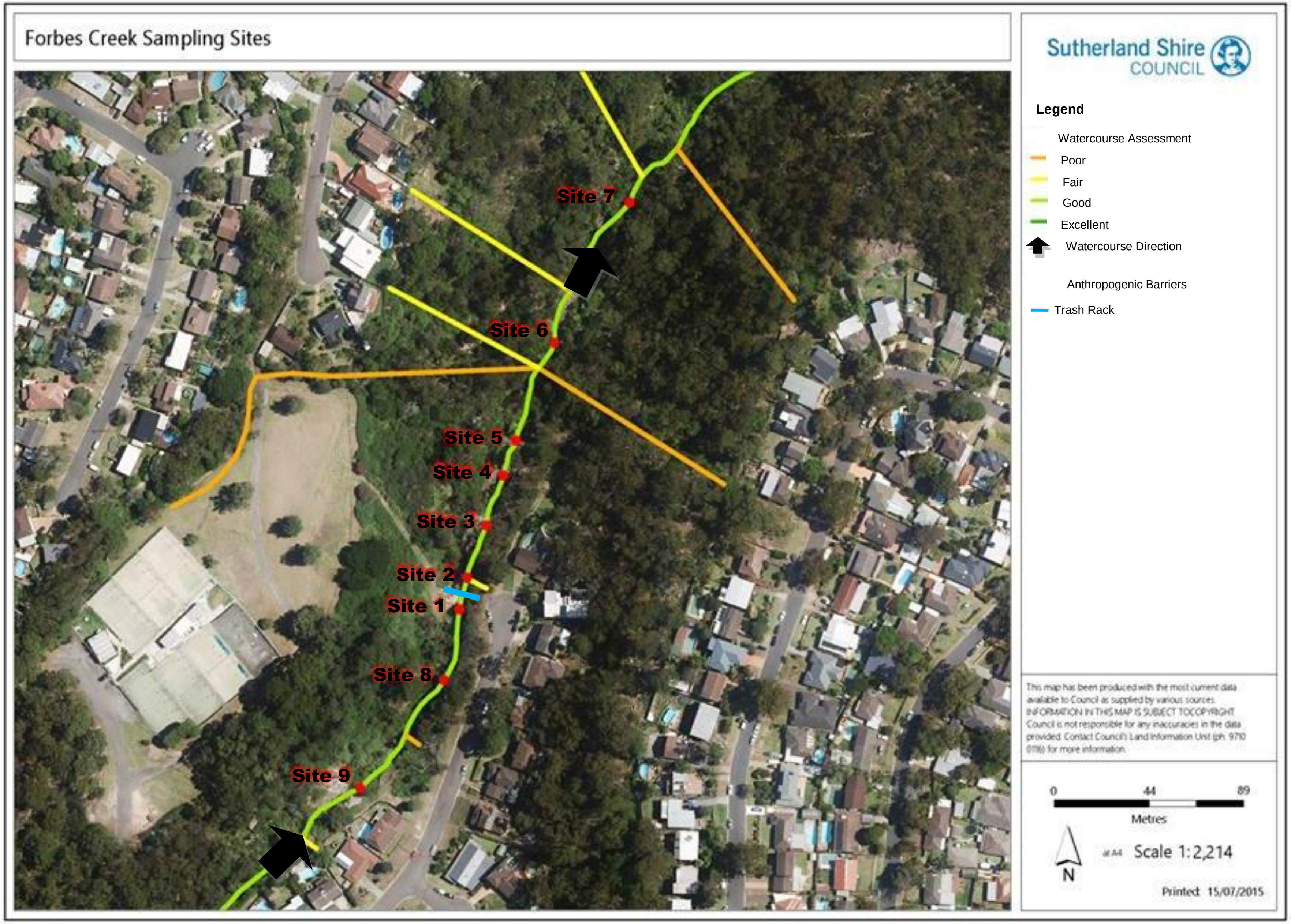

Plate 3.2 Forbes Creek Sampling Site Locations.

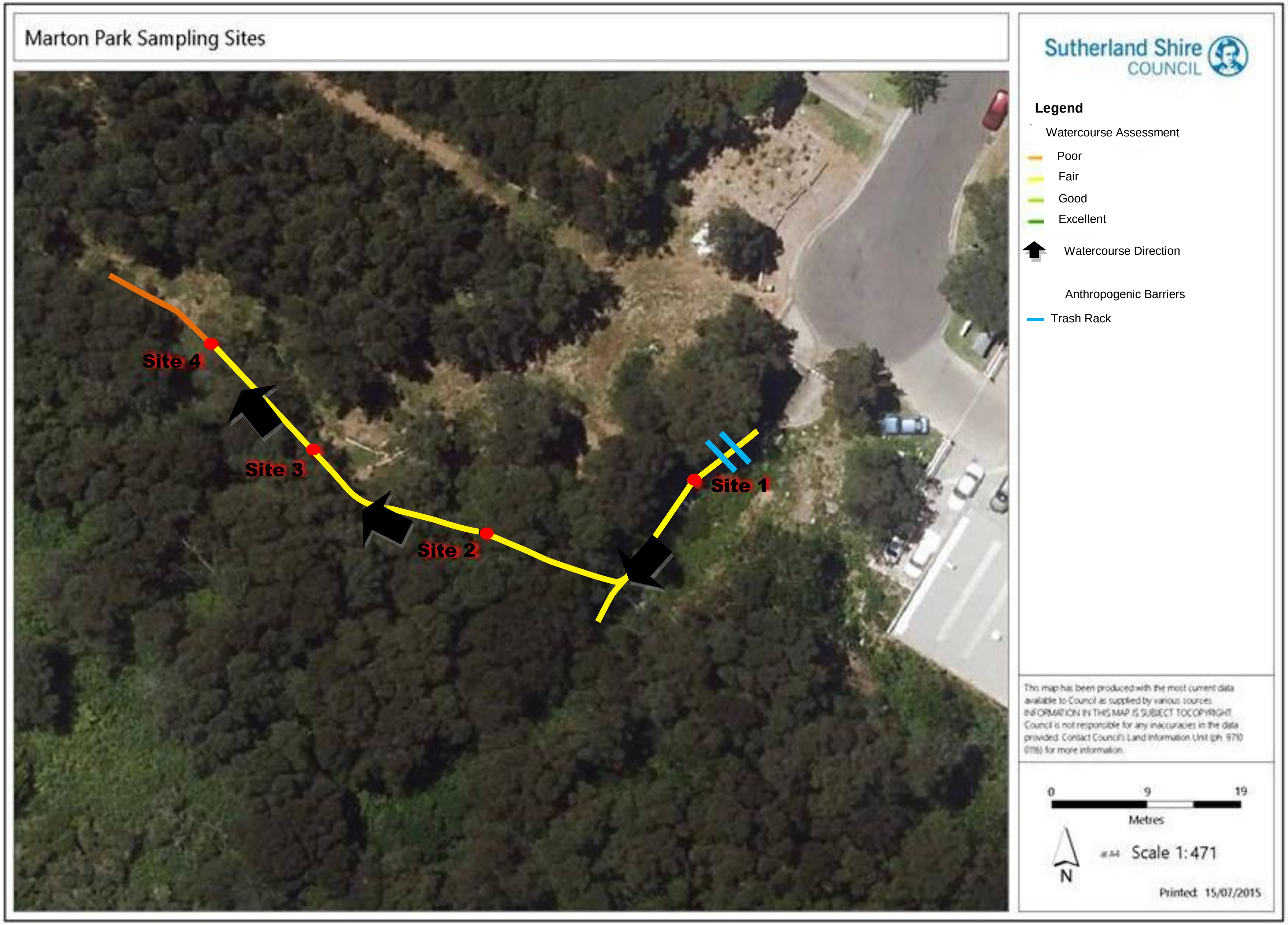

Plate 3.3 Marton Park Sampling Site Locations.

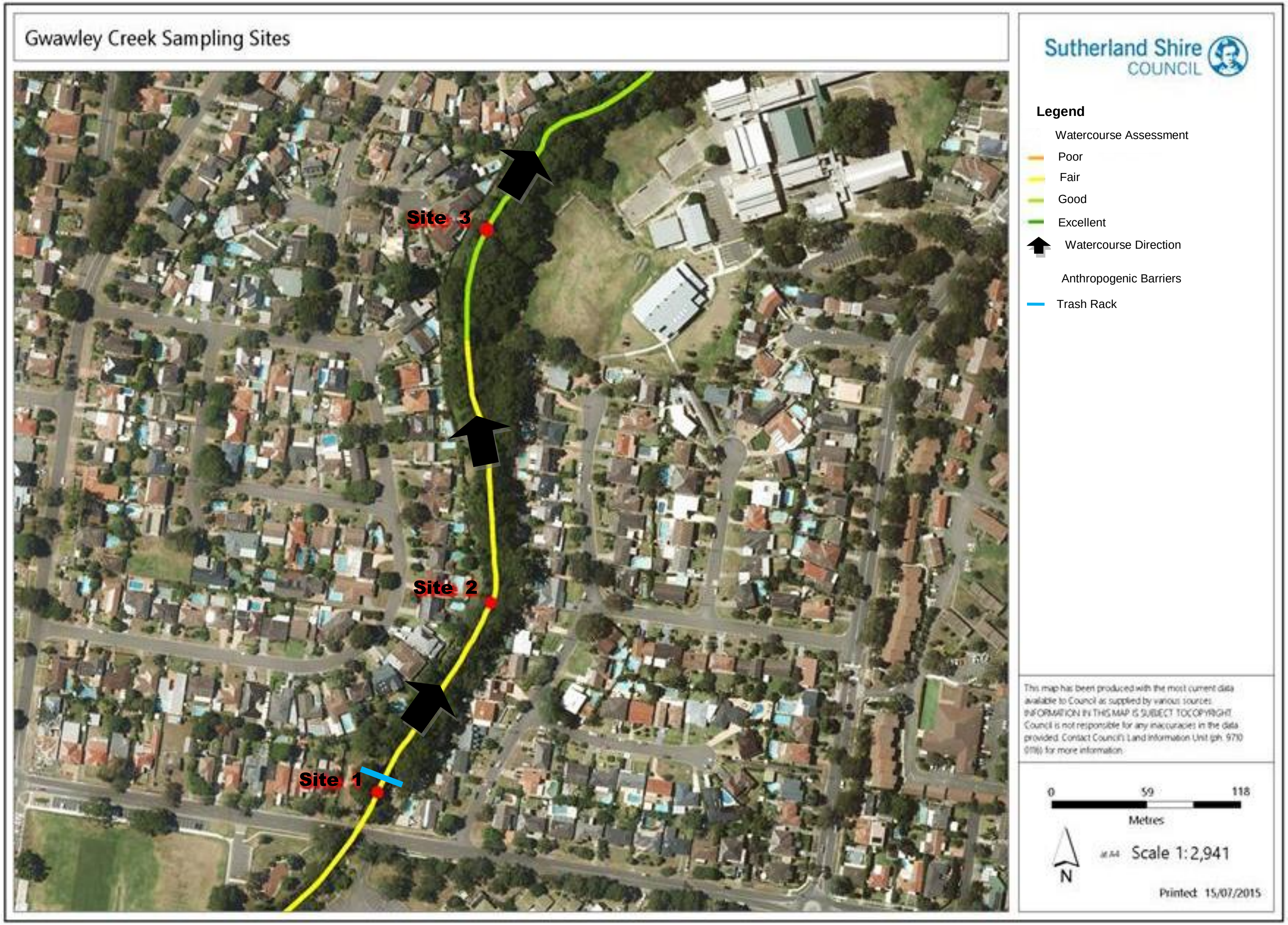

Plate 3.4 Gwawley Creek Sampling Site Locations.

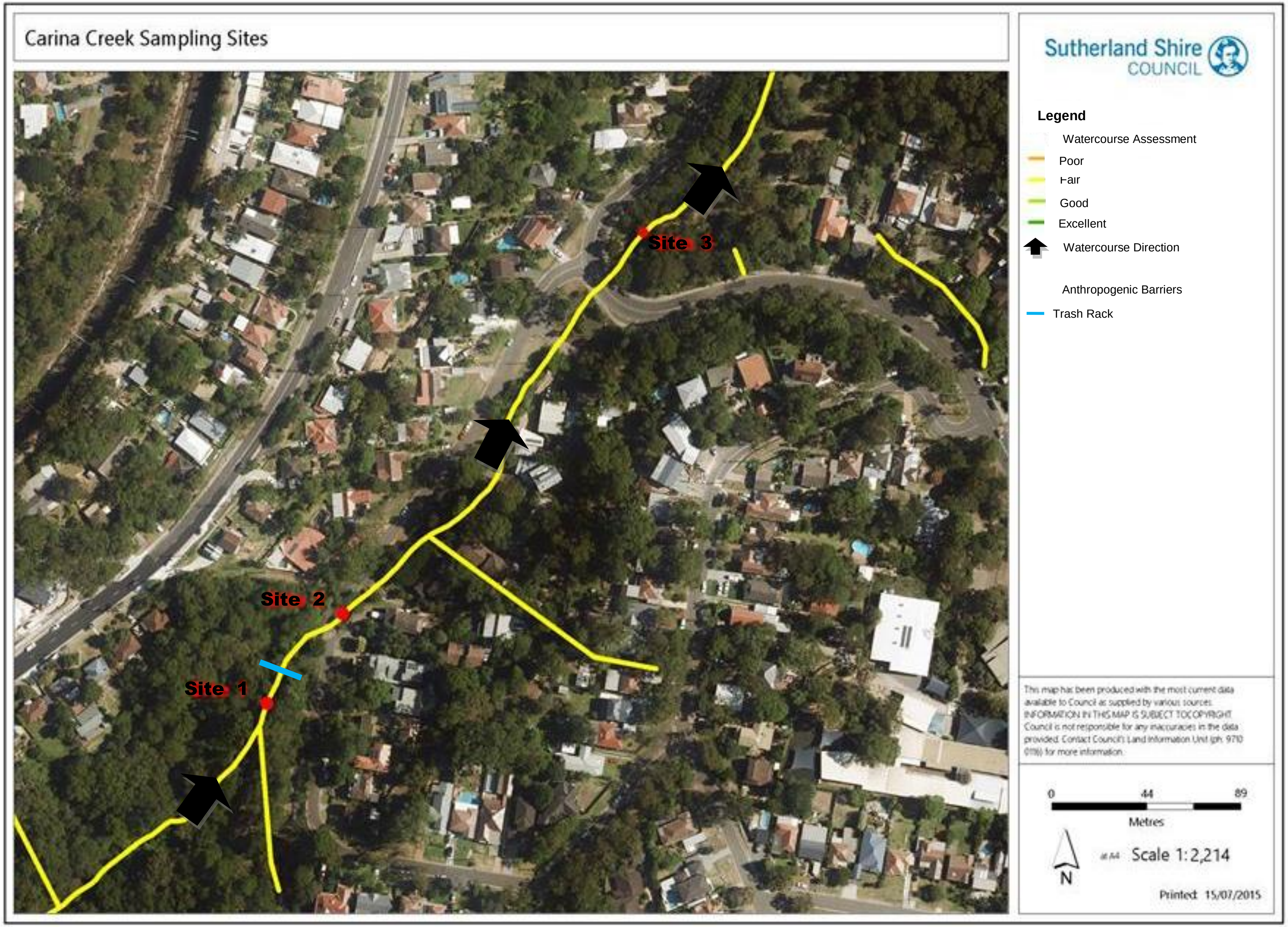

Plate 3.5 Carina Creek Sampling Site Locations.

3.1.2 SPECIMEN SAMPLING

Specimen Collection

Individuals were collected using a Shimano Environet FML000 landing net with mesh size 4x4mm. Individuals were subsequently scooped out of the net using a container and placed into aerated buckets to ensure minimal stress to the individuals. Fresh water was added approximately every 10 minutes to the buckets to ensure sufficient oxygen supply. Any fish that was seen to be under stress was carefully removed with a hand net and placed in a separate aerated bucket to be monitored. If the fish still showed signs of stress then it was placed back into the creek without tagging or measurement. In obtaining individuals for tagging purposes, active netting was targeted at the furthest point upstream where *Gambusia holbrooki* were observed. At Forbes Creek and Marton Park, this was in Site 1 (Plate 3.2 and 3.3). Active netting was haphazardly distributed within Site 1 for both sites, and occurred until enough fish were obtained for tagging (≈350 per site). Specimen collection and tagging occurred over two days for each site. Tagging methodology is outlined below.

Tagging and Release

Individuals were tagged using a Visible Implant Elastomer (VIE), which are coloured or fluorescent tags that are implanted beneath translucent tissues and remain externally visible. Fish were individually netted from the aerated buckets with a hand net and placed on a damp, stable surface for tagging. Tags were inserted on the dorsal surface, between the head and dorsal fin on the left side (Figures 3.5 and 3.6) using syringes filled with the fluorescent polymer dye. This procedure lasted less than 60 seconds per fish; and if for any reason the tagging procedure was not completed within this time frame, fish were replaced back into the water to recuperate for 60 seconds prior to a second measuring attempt. Prior to tagging, each individual was measured using a vernier caliper and sex was determined and recorded. Any individuals that were less than 20mm were not tagged, due to an observed increased mortality rate of fish less than this length.

Two different coloured tags were used in each site. Fish with red tags were released upstream of the trash racks in both sites, and fish with yellow tags were released downstream of the trash rack in both sites (Plate 3.6 and 3.7). Approximately half of the

≈350 fish at each site were tagged with red dye, and the other half with yellow. After tagging and measuring, individual fish were immediately released to their designated site based on their tag colour.

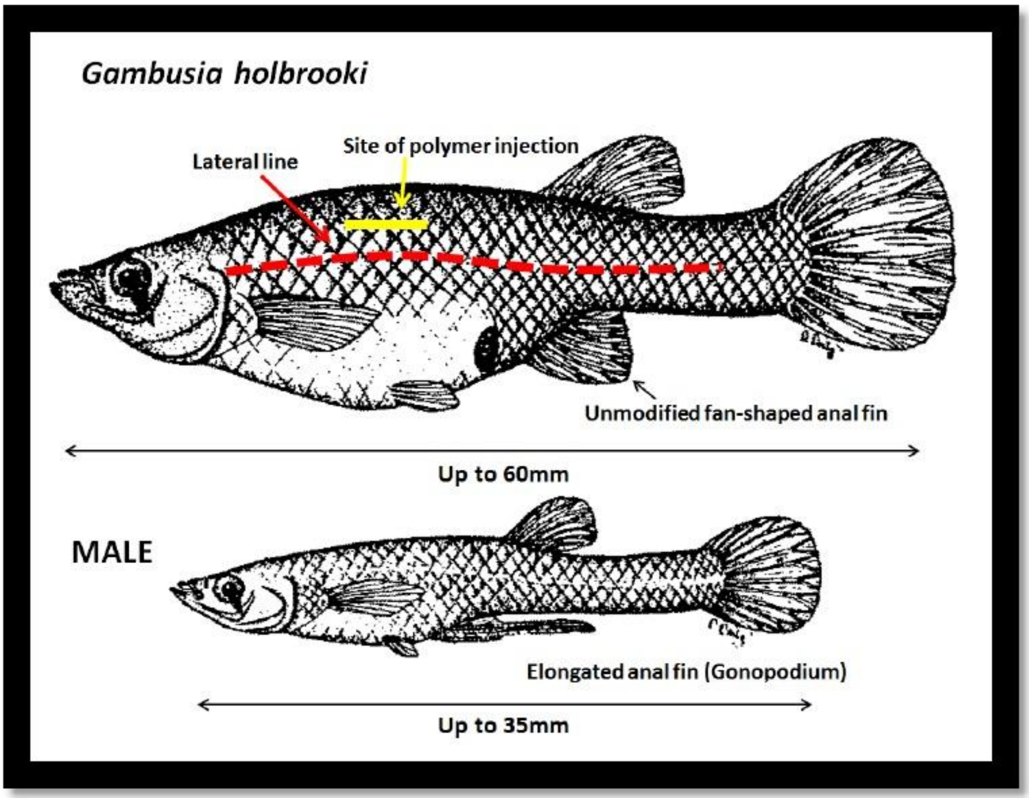

Figure 3.5. Anatomical differences between male and female G. holbrooki indicating the site of fluorescent polymer tags. Image taken from Matthews, 2013.

Figure 3.6. Tagged and measured **Gambusia holbrooki** ***with red tag along the dorsal surface.***

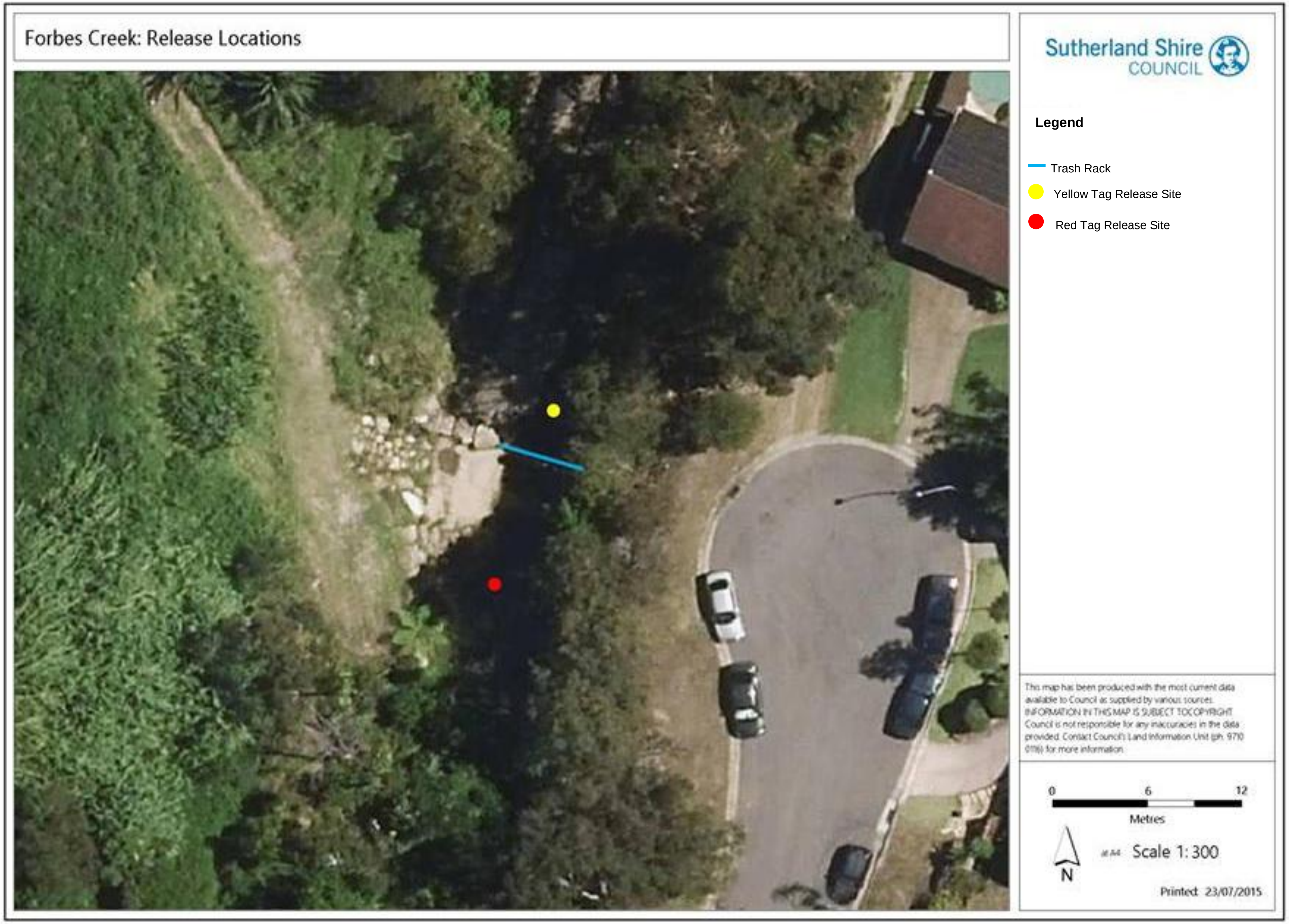

Plate 3.6 Forbes Creek release sites for tagged Gambusia holbrooki.

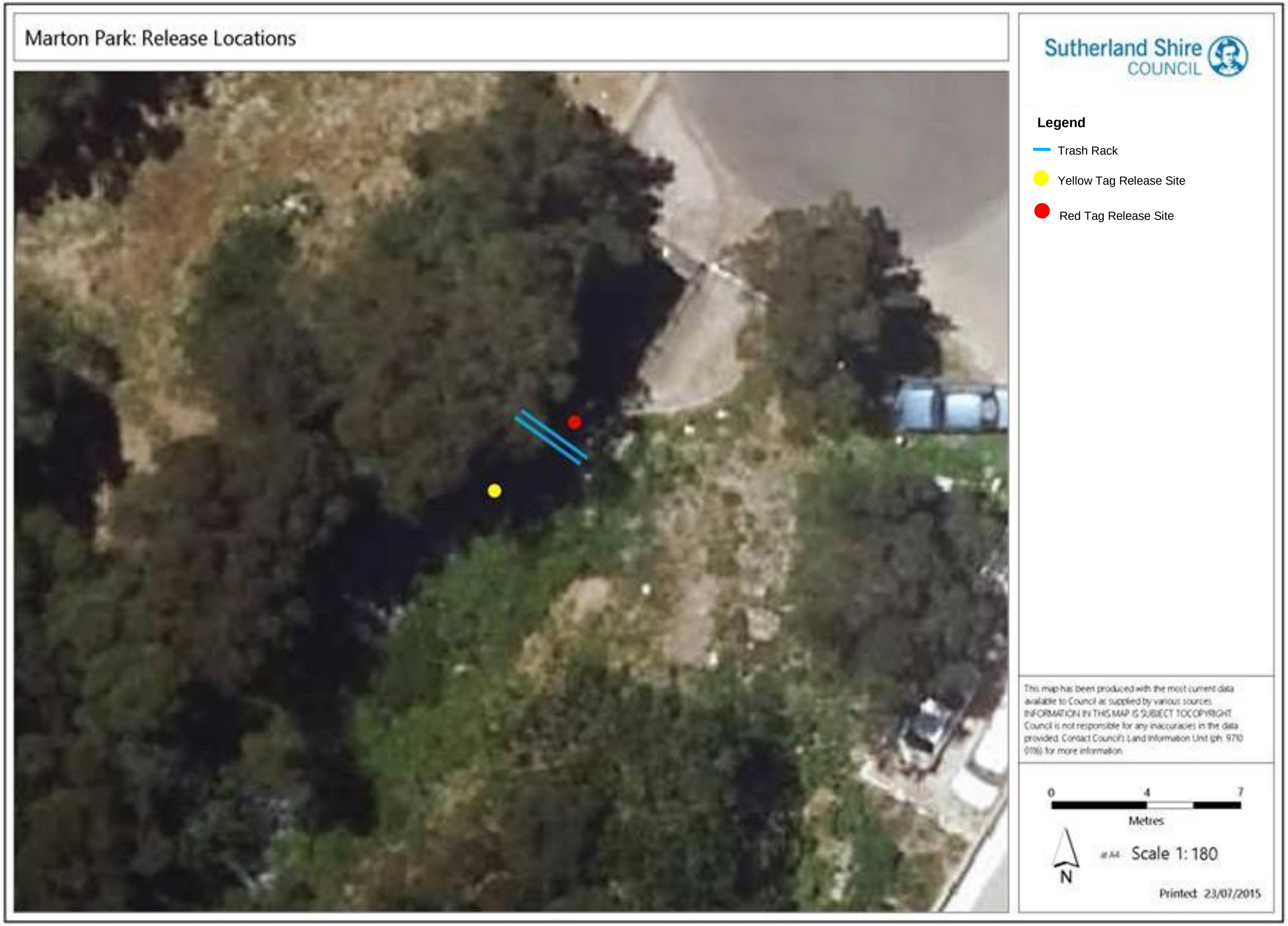

Plate 3.7 Marton Park release sites for tagged Gambusia holbrooki.

Re-sampling and Re-capturing

Sample sites within Forbes Creek and Marton Park were determined by the accessibility of the site, and the suitability of the site for *Gambusia holbrooki* as outlined by existing literature. The parameters that determined whether areas were suitable or not were based on flow rate, depth of the creek and presence of vegetation. *G. holbrooki* are unable to survive long term in extremely shallow, ephemeral zones less than 3mm deep and can only persist in low flow zones where vegetation is present (Jackson and Bamford, 2011). Therefore, selecting sites based on these preferences is the most efficient way to increase the probability of re-sampling tagged *G. holbrooki*. The number of sample sites selected in each location was primarily determined by the accessible length of the creek via fire trails and cleared land. The difference in site numbers between Forbes Creek (9 sites) and Marton Park (4 sites) (Plate 3.3 and 3.4; Appendix 2 and 3) is also due to differences in the total distance that the watercourse extends. Site boundaries were determined by physical barriers such as fallen trees, built up debris, trash racks, large boulders and knickpoints, and therefore the dimensions of each site differ (Appendix 6).

During the re-capture stage of sampling, each site was sampled weekly for 9 weeks subsequent to tagging, spanning from June 2 – August 11. Within each site, ten 15 second net sweeps were randomly conducted, sweeping approximately 1m around the netter's standing position (Figure 3.7). In areas where it is too unstable or unsafe to stand in the creek and conduct a sweep, the sweep was taken standing along the bank. After each sweep, *Gambusia holbrooki* were collected from the net using a container and put into an aerated bucket where they are counted and inspected for any tags. All tagged and untagged fish were recorded. However, if any tagged fish were re-captured, their measurements and sex were recorded before re-releasing them. Any tadpole and macro-invertebrate captures were identified and recorded as well. *Gambusia holbrooki,* tadpoles and macro-invertebrates were all stored in separate buckets in between sweeps to avoid potential predation and excess stress. This is done for all 10 sweeps, and only after the last sweep has been conducted were all individuals are released within the site.

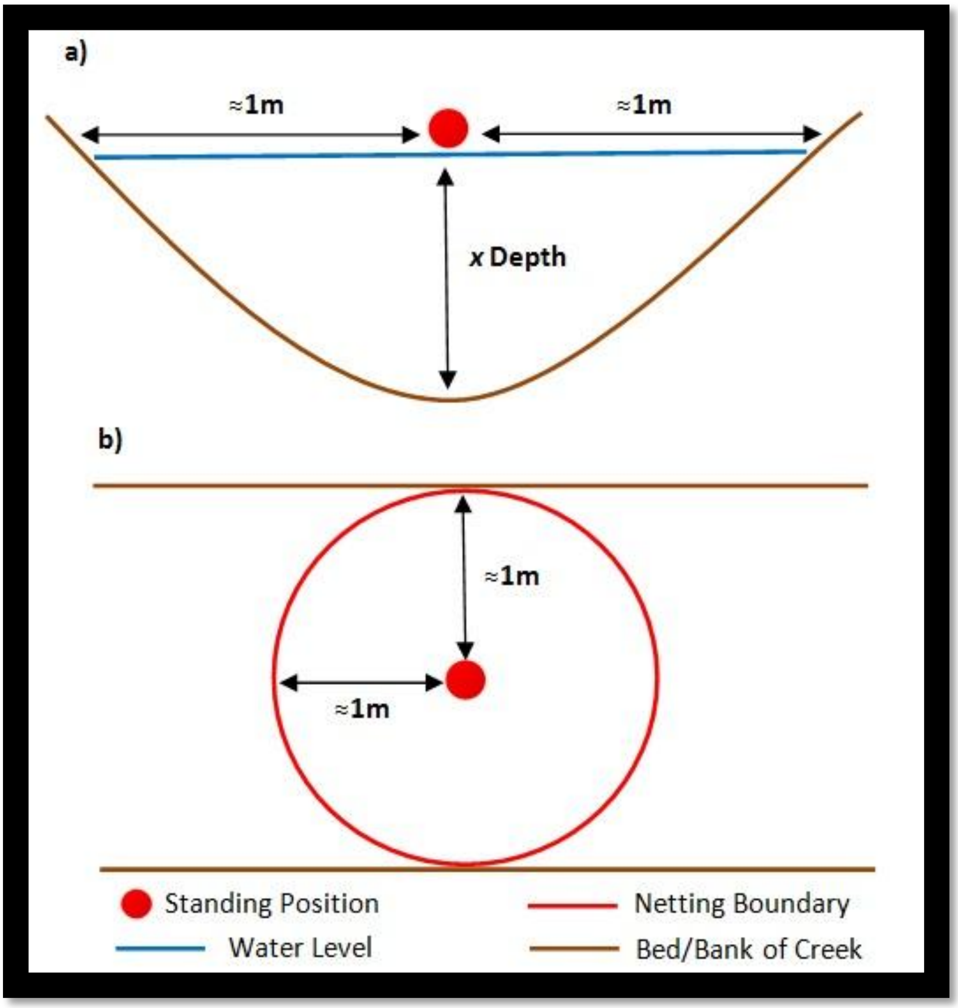

Figure 3.7 a) Cross sectional view of the sweeping technique in water body. b) Aerial view of the sweeping technique implemented during the re-capture stage of sampling.

3.1.3 RE-ESTABLISHMENT ESTIMATES

To determine the re-establishment of *Gambusia holbrooki* at Carina Creek and Gwawley Creek, visits to both these locations were conducted weekly. Random sweeping events were performed in areas where accessibility is not limited between Site 1 and Site 3 (Plate 3.4 and 3.5) in both locations. Areas that were targeted for sample sweeps were those zones where *G. holbrooki* had seen to previously inhabit, and those sites that had low flow and presence of vegetation that were deeper than 3mm. The presence or absence of *G. holbrooki* at both sites was recorded after a minimum of 10 sweeps at each location.

3.1.4 PHYSICO-CHEMICAL ANALYSIS

At all of the sampling locations, both water quality measures and percent vegetation cover were determined as part of the physico-chemical analysis at each sample site within those locations (Appendix 2-5). This was done weekly for 9 weeks. This was conducted using a TPS 90FL-T water quality logger which tested for: turbidity (NTU), dissolved oxygen (ppm), electrical conductivity (uS), temperature (°C) and pH. The water quality probes were submerged for approximately 3 minutes at each site, and were thoroughly rinsed with distilled water and dried between each site to avoid cross contamination and minimise systematic error. I sought to determine the range of physico-chemical conditions that *Gambusia holbrooki* have been able to tolerate, as well as determining the similarity between environmental parameters at those locations where *G. holbrooki* are present and absent.

Percentage vegetation cover was estimated at each location in reference to bank and macrophyte vegetation. This was estimated using the transect-quadrat methodology. Two 10m transects were randomly positioned along either side of the bank at each site within each location. Ten 1x1 meter quadrats within each transect were haphazardly placed along each bank transect according to Figure 3.8, and percentage cover was quantified for each quadrat. To minimise subjective error when estimating percent cover, a reference guide was used to help determine percentage cover in a plot area (Appendix 7).

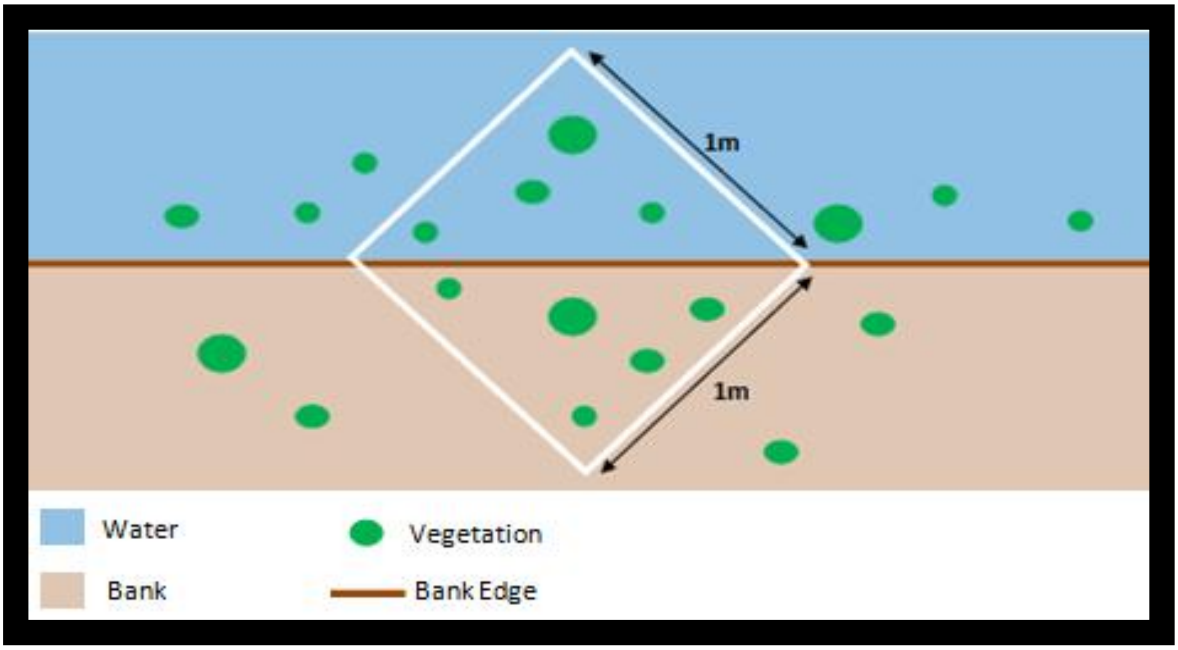

Figure 3.8 Quadrat sampling design along a transect, used for determining percentage vegetation cover of each sampling site within each study location.

3.2 DATA ANALYSIS

3.2.1 UNIVARIATE ANALYSIS

All univariate statistics were performed using JMP Pro 11 (JMP) statistical software package and all graphs were constructed using Microsoft Excel 2007. Chi-squared analyses, linear regression models and relative abundance measures were undertaken as part of the univariate analysis.

Chi-square tests were performed using the significance level $p<0.05$ for all tests. Using JMP, a chi-squared analysis was performed to determine any significant differences between Marton Park and Forbes Creek in relation to the frequency of tagged fish found upstream of the trash rack compared to those found downstream of the trash rack. Similarly, chi-squared tests also determine whether the frequency of red tagged individuals found at areas other than the site of release, were significantly different to that of yellow tagged individuals. This was conducted for both Marton Park and Forbes Creek separately. A test for male/female equality was also conducted to determine whether the frequency of males and females were significantly different for red and yellow re-captured individuals at Marton Park. This test did not need to be conducted for Forbes Creek, due to the majority of re-captures being female.

Using JMP, simple linear regression models were conducted using Log (x+1) transformation on all data to normalise the datasets. A model was performed on body size (mm) and distance travelled (m) of tagged individuals, to determine whether there was a relationship between their body size and how far they were observed to have travelled at Marton Park. This analysis could not be conducted at Forbes Creek due to the tagged individuals only being observed to have travelled to one particular site. A linear regression model was also conducted to determine whether there was a relationship between the amount of rainfall (mm) and the observed distance travelled by tagged individuals at Marton Park. This was also unable to be performed for Forbes Creek due to the nature of the data.

Relative abundance measures were determined by taking the mean of 9 weeks of catch-per-unit-effort abundance data for each site within each location at Marton Park and Forbes Creek. This data was then categorised into groups (1-50, 50-100 and 100+ individuals) to then be used to compare abundance between Marton Park and Forbes Creek and their respective sites. This was done solely to make a comparison between the abundance between Marton Park and Forbes Creek, in addition to making comparisons between the sites within these locations. Providing that sampling is conducted with low bias, high precision and standardisation of techniques, it is justifiable to use this methodology to compare relative abundance between sites and locations in this study.

3.2.2 MULTIVARIATE ANALYSIS

All multivariate statistics were analysed using the PRIMER 7 statistical software package.

Environmental data was incorporated into a multivariate analysis, with factors added for "presence/absence" of *Gambusia holbrooki* and "location/site". This was done with the aim to see if physico-chemical parameters significantly differed between those locations and sites where *G. holbrooki* was present compared to where they were absent. A Euclidean Distance resemblance matrix was then applied to the data and an MDS plot was constructed to examine the grouping of variables within the data. The environmental variables examined included: pH, electrical conductivity (uS), turbidity (NTU), dissolved oxygen (ppm), temperature (°C) and percentage cover of bank and macrophyte vegetation (%). A Permanova main test was then performed using 999 permutations of the data. The factors in the Permanova included: presence (fixed) and location (nested in presence, random). A pair-wise test was then conducted to test for significant differences between the 4 levels within "location" (Marton Park, Forbes Creek, Carina Creek and Gwawley Creek).

4. RESULTS

4.1 DISPERSAL PATTERNS AND EXTENT

Over 9 weeks of re-sampling, a pooled total of 68 tagged *Gambusia holbrooki* were recaptured at Forbes Creek out of a total of 310 tagged individuals; and 45 were re-captured at Marton Park of a total of 321 individuals (Figure 4.1). Out of the 68 tagged individuals at Forbes Creek, 21% of those re-captured were yellow tagged individuals that were released downstream of the creeks trash rack, and the remaining 79% were red tagged individuals that were released upstream of the trash rack (Figure 4.1) (Plate 3.6). Of the 45 tagged individuals at Marton Park, 67% of those re-captured were yellow tagged individuals that were released downstream of the wetlands trash racks, and the remaining 33% were red tagged individuals that were released upstream of the trash rack (Figure 4.1) (Plate 3.7). There is a significant difference in the frequency of recapture below versus above the trash rack (yellow versus red tagged individuals respectively) between these sites (χ^2=24.18, DF=1, P=0.00001) (Figure 4.1).

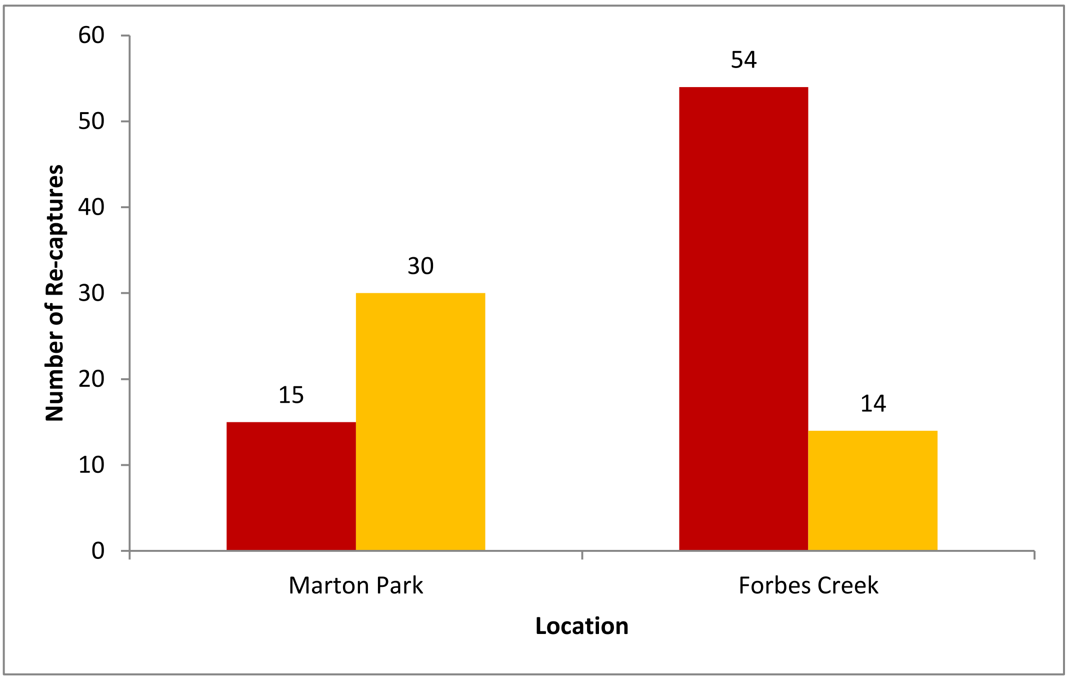

Figure 4.1 Total number of re-captured tagged individuals at both Marton Park and Forbes Creek over the experimental time frame (9 weeks). Red and yellow columns represent red (upstream) and yellow (downstream) tagged individuals respectively.

4.1.1 FORBES CREEK

Patterns of Recapture

At Forbes Creek there was no observed movement of red tagged individuals upstream or downstream of the release site (Site 1) during the experimental time frame (June 2 – August 11). All of the red tagged individuals were re-captured at the site of release (Plate 4.1). However, it was observed that yellow tagged individuals had dispersed from their original release location (Site 2) and *all* observed individuals had travelled between 51-60m from their original release location to Site 4 (Plate 4.1). At this location, the frequency of red tagged fish found at areas other than the site of release was significantly different to that of yellow tagged fish, which were found in sites other than their release location (χ^2=68, DF=1, P=0.0001). Yellow tagged individuals only first appeared in sampling and re-capture efforts 5 weeks after their initial release (Figure 4.2). At this site the majority of re-captured individuals were female (62%), with a high proportion of tagged individuals ranging from 20-30mm in size (Figure 4.3). This is reflective of the size profile of individuals that were tagged prior to release (Figure 4.4). The majority of these individuals were of 20-30mm in size, with a high proportion of these tagged individuals also being female (72%).

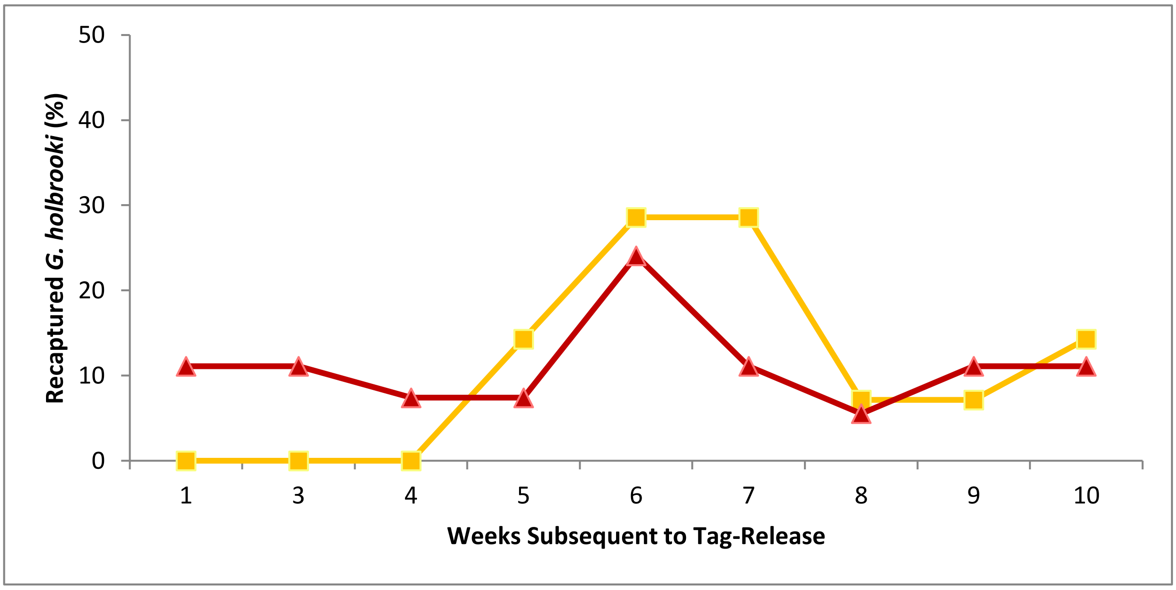

Figure 4.2 Weekly re-capture profile of red and yellow tagged individuals over the experimental time period. Depicts the percentage of tagged* G. holbrooki *captured each week at Forbes Creek. Sampling was not conducted in Week 2.

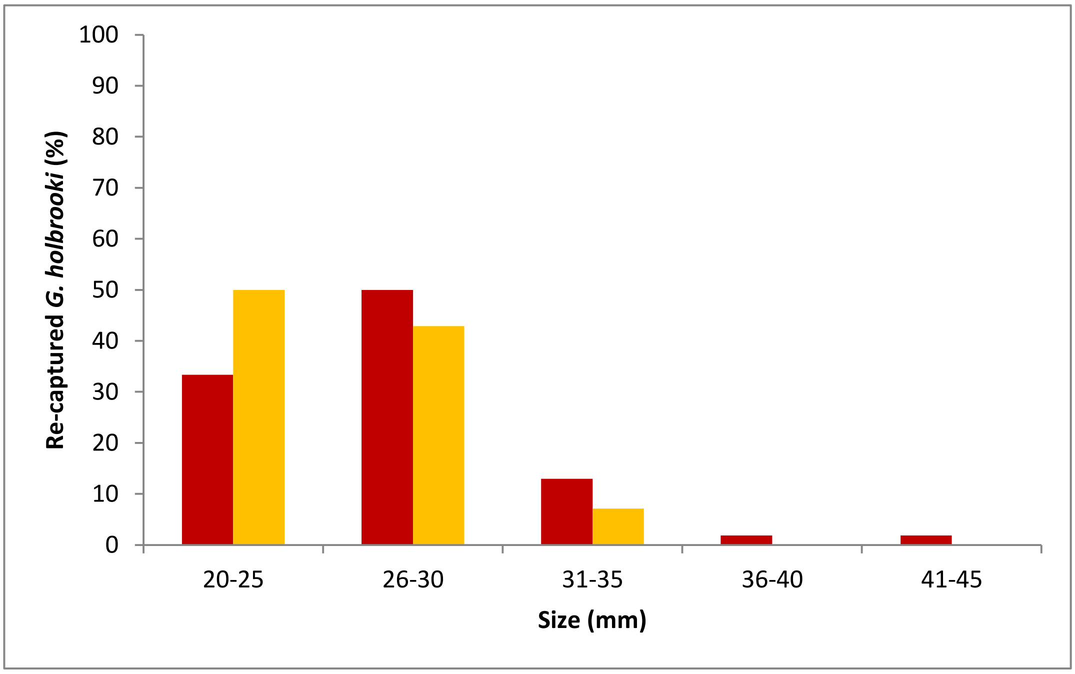

Figure 4.3 Size profile of re-captured yellow and red tagged individuals at Forbes Creek over the experimental time frame.

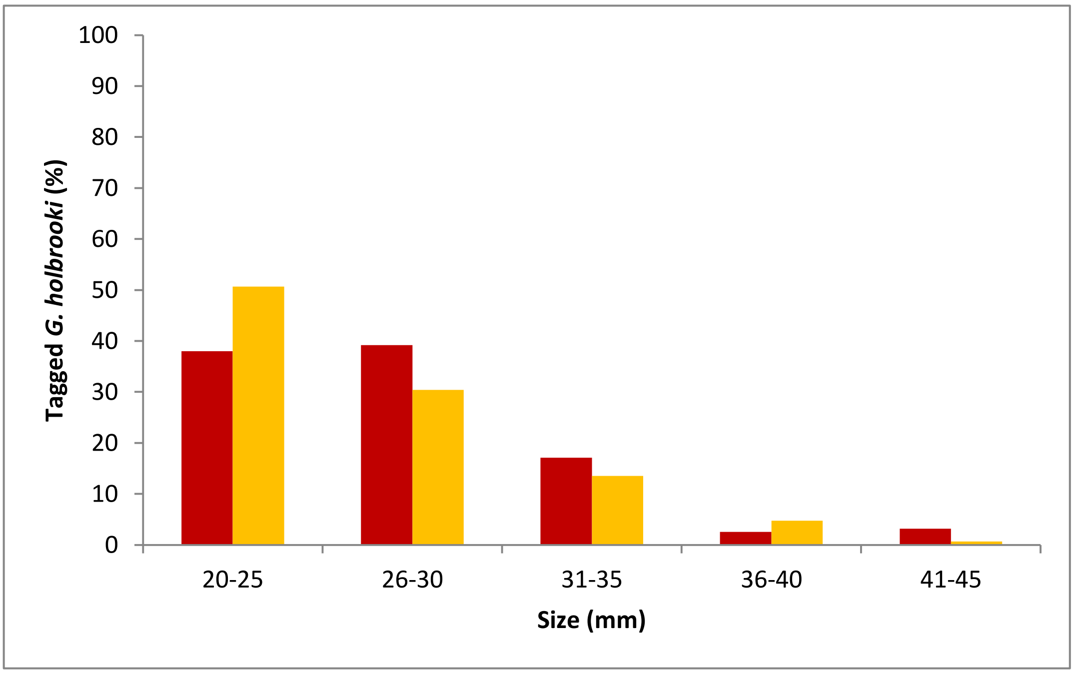

Figure 4.4. Size profile of all yellow and red tagged individuals at Forbes Creek prior to release.

Recapture and Physical Barriers

The major physical barriers present at this site include: trash racks, fallen trees, knickpoints and debris build up (Plate 4.2). Of the natural physical barriers, there are 5 observed knickpoints of various channel slope gradients and 2 large fallen trees (Plate 4.2). All yellow tagged individuals that were re-captured were found downstream of two knickpoints (Site 4) from their initial release location (Site 2). No significant observations regarding fallen trees were observed in relation to the dispersal patterns of *G holbrooki* at this site.

Of the anthropogenic barriers, there is one trash rack and build-up of debris between Sites 1 and 2, and Sites 5 and 6 respectively (Plate 4.2). Throughout the experimental time period, no trash from the trash rack was removed or cleared. It was found that from Week 5 onwards, all yellow tagged individuals that were re-captured were found downstream of two knickpoints (Site 4) from their initial release location (Site 2). None of the red-tagged individuals however were observed to have moved from Site 1 where they were initially released.

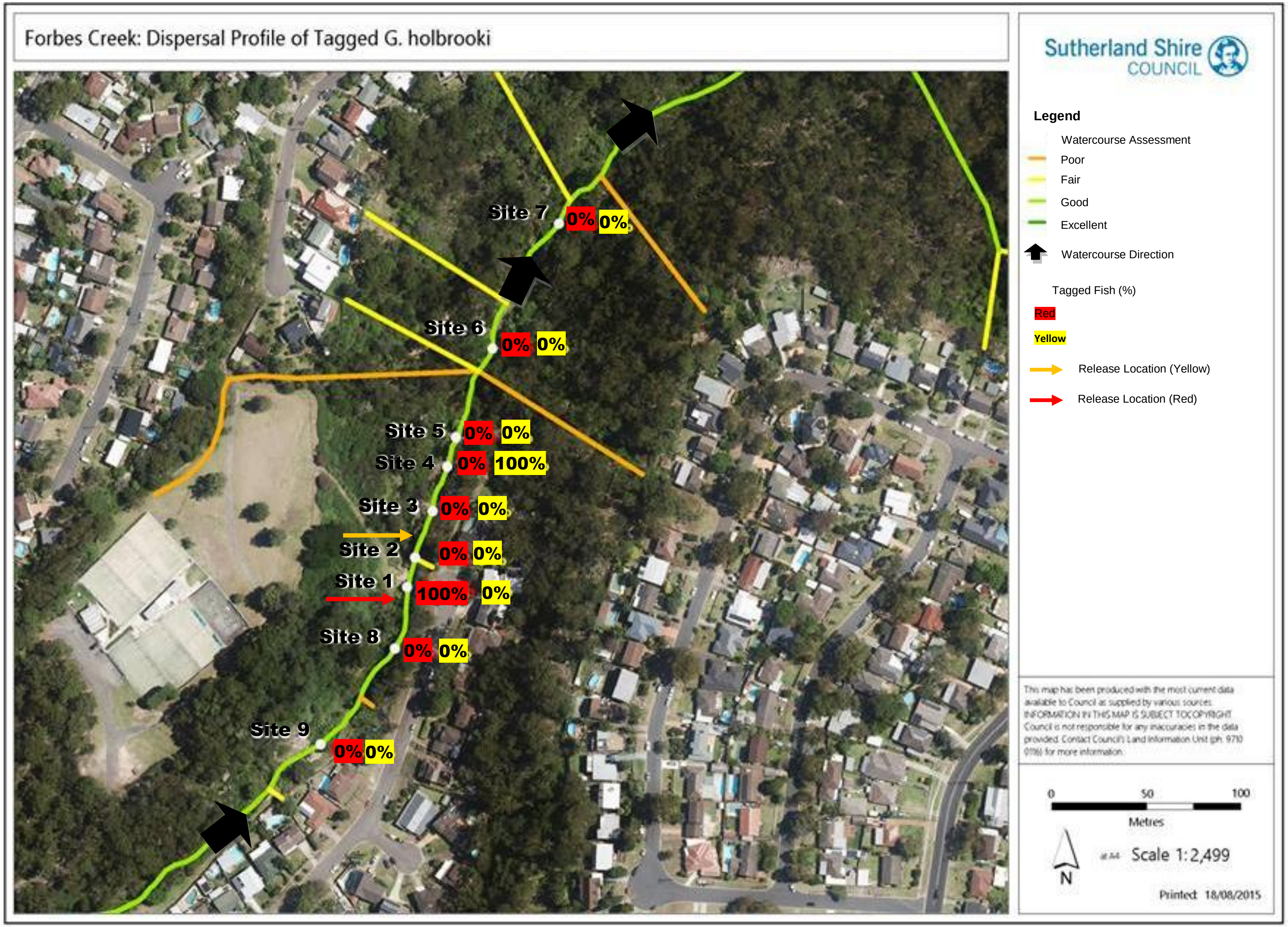

Plate 4.1 Dispersal profile of both yellow and red tagged G. holbrooki that have been recaptured at Forbes Creek. Percentages indicate the proportion of tagged G. holbrooki recaptured at each site.

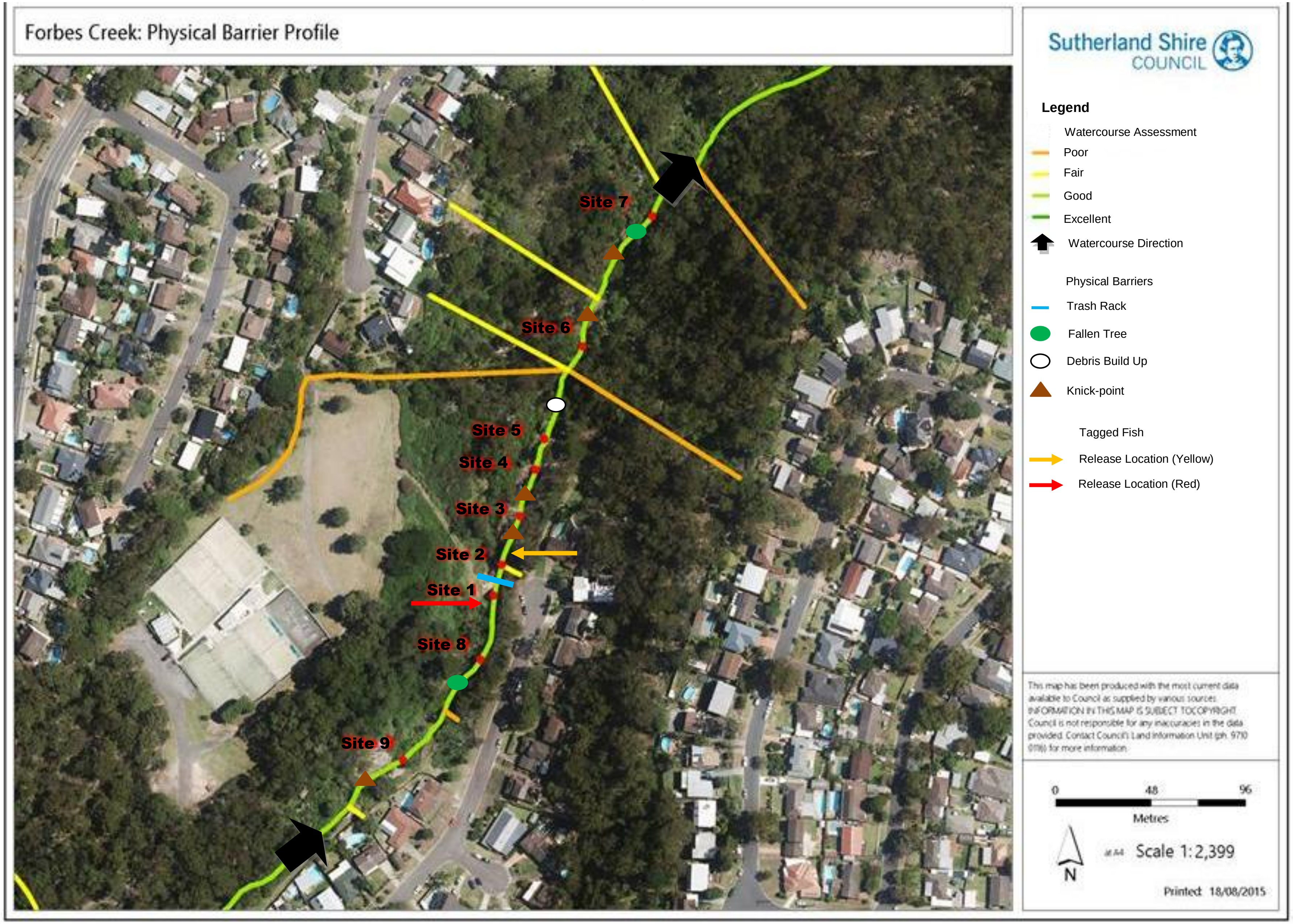

Plate 4.2 Physical barrier profile of Forbes Creek. Depicts major barriers that could potentially hinder the dispersal of G. holbrooki.

4.1.2 MARTON PARK WETLAND

Patterns of Recapture

At Marton Park Wetland there was no observed movement of yellow tagged individuals upstream or downstream of the release site (Site 1) during the experimental time frame (June 2 – August 11)(Figure 4.5) (Plate 4.3). All of the yellow tagged individuals were re-captured at the site of release. However, it was observed that red tagged individuals had dispersed from their original release location (upstream of trash racks). The majority of red tagged individuals were re-captured in Site 1 (80%), followed by Site 3 (13%) and Site 2 (7%) (Plate 4.3). At this location, the frequency of red tagged fish found at areas other than the site of release was significantly different to that of yellow tagged fish, which were found in sites other than their release location (χ^2=68, DF=1, P=0.0001). None were observed in Site 4. The majority of the observed red tagged individuals travelled 1-10m from their initial release site (60%) with smaller numbers travelling 11-20m (20%), 31-40m (7%) and 41-50m (13%) (Figure 4.5). Both yellow and red tagged individuals were captured during every week of sampling after their initial release (Figure 4.6). At this site an approximately equal proportion of males and females were recaptured (49% male, 51% female) (χ^2=0.18, DF=1, P=0.67), with a high proportion of tagged individuals ranging from 20-30mm (Figure 4.7). This is reflective of the size profile of individuals that were tagged prior to release (Figure 4.8). The majority of these individuals were of 20-30mm in size, with a high proportion of these tagged individuals also being female (60%). There also seems to be no strong correlation between body size, distance and sex in determining the movement and migration of individuals at this location (R^2=0.13, r=0.36, P=0.19) (Figure 4.9).

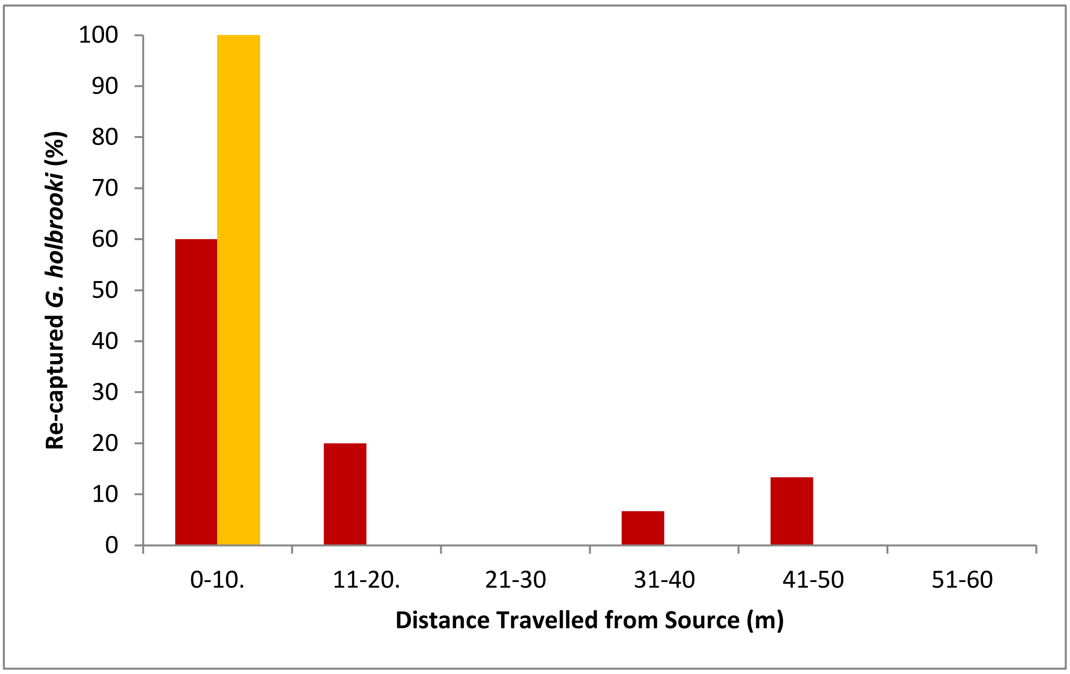

Figure 4.5 Observed dispersal distance travelled by red and yellow tagged individuals that have been re-captured at Marton Park.

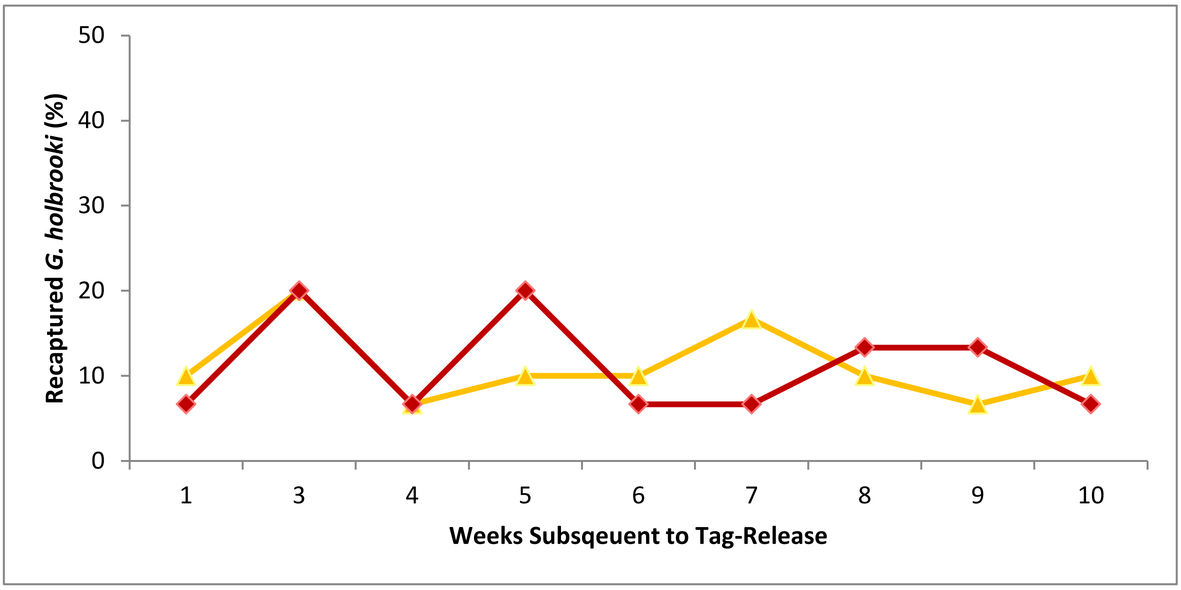

Figure 4.6 Weekly re-capture profile of red and yellow tagged individuals over the experimental time period. Depicts the percentage proportion of tagged G. holbrooki captured each week at Marton Park. Sampling was unable to be conducted in Week 2.

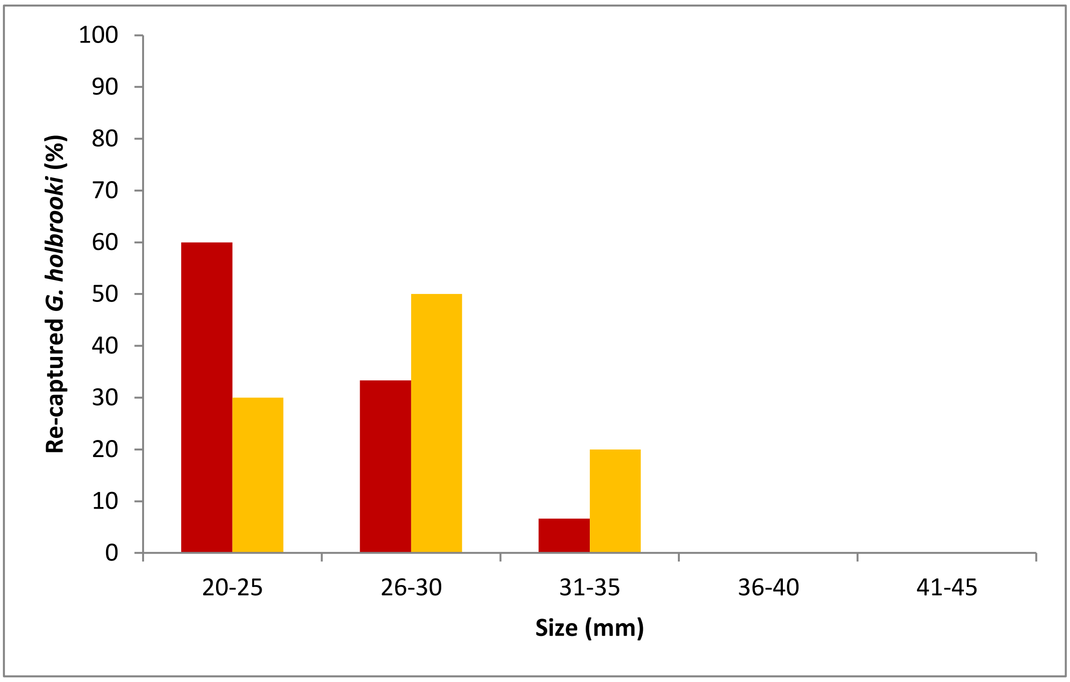

Figure 4.7 Size profiles of re-captured yellow and red tagged individuals at Marton Park over the experimental time frame.

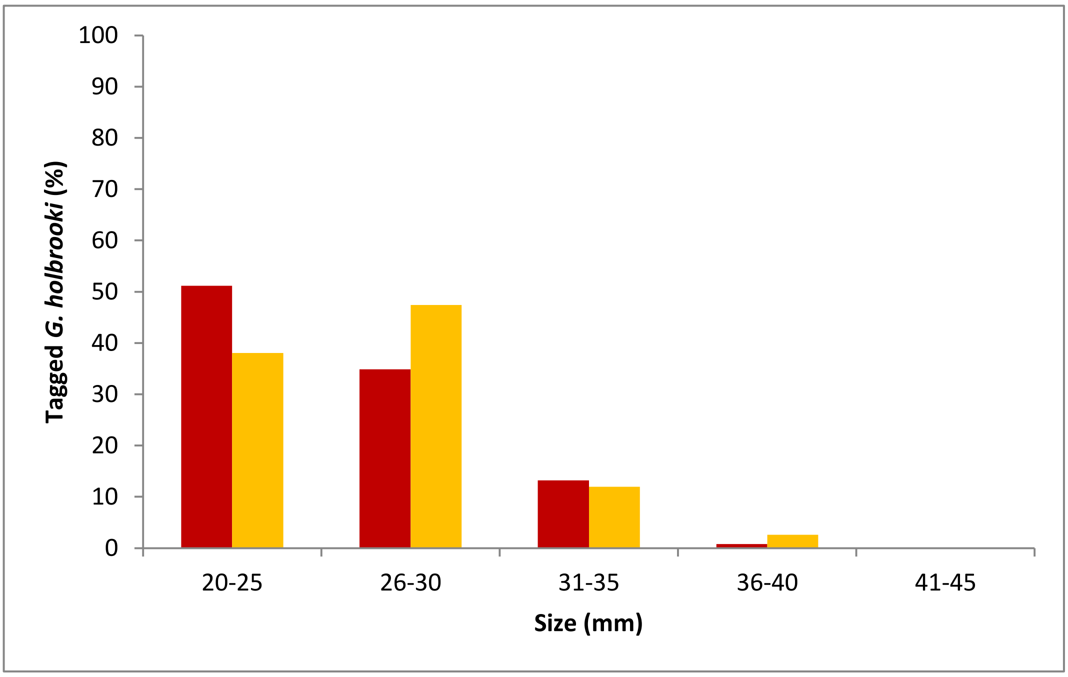

Figure 4.8 Size profile of all yellow and red tagged individuals at Marton Park Reserve prior to release.

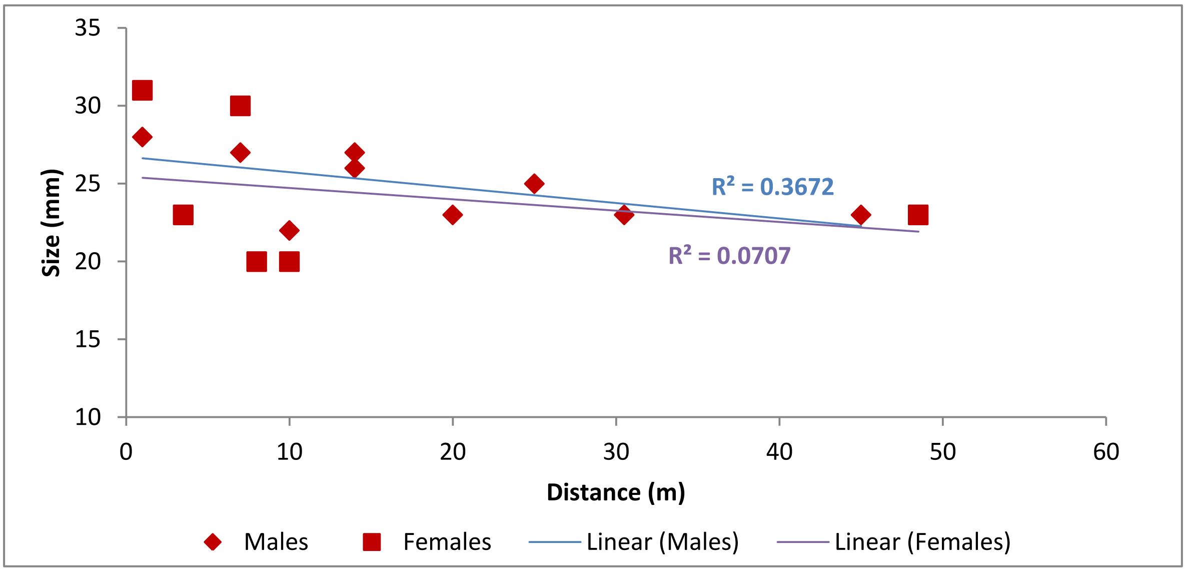

Figure 4.9 Scatter plot of red tagged individuals at Marton Park. Depicts the distance travelled by individuals relative to size and sex; female ( ) and male ( ) represented respectively.

Recapture and Physical Barriers

The main physical barriers that were profiled at this site as potential influencers on *Gambusia holbrooki* dispersal at this site included: trash racks, fallen trees and build-up of debris (Plate 4.4). There were 2 fallen trees which potentially act to limit the hydraulic connectivity, in addition to build up of organic debris such as leaf litter, branches and twigs (Plate 4.4). It had been observed that these natural 'barriers' did not completely exclude *G. holbrooki*, due to the presence of tagged individuals found in Sites 1-3 (Plate 4.3).

Anthropogenic barriers such as trash racks and presence of petrochemicals on surface water were quantified at this location upstream of Site 1 (Plate 4.4). Throughout the experimental time period there were negligible amounts of trash that accumulated in the trash rack, allowing for the movement of *Gambusia holbrooki* through the trash rack with minimal physical hindrance. The movement of red tagged individuals downstream of the trash rack at this site compared to Forbes Creek provides evidence of this. The presence of petrochemicals within this location was concentrated at the drainage inlet site <5m upstream of the trash racks. The presence of petrochemicals was not observed to have deterred *G. holbrooki* from persisting in that zone of the water body.

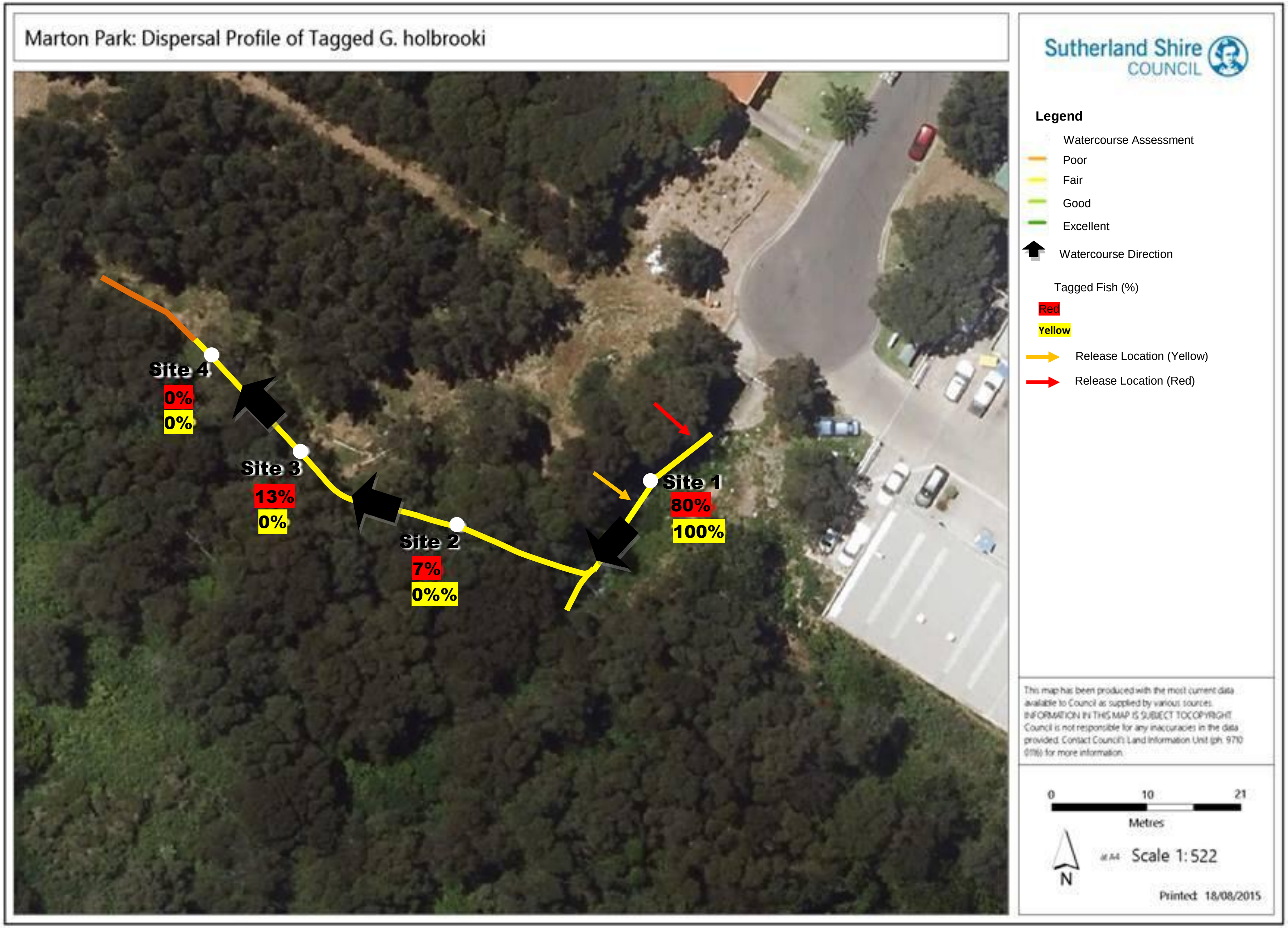

Plate 4.3 Dispersal profile of both yellow and red tagged G. holbrooki that have been recaptured at Marton Park. Percentages indicate the proportion of tagged G. holbrooki recaptured at each site.

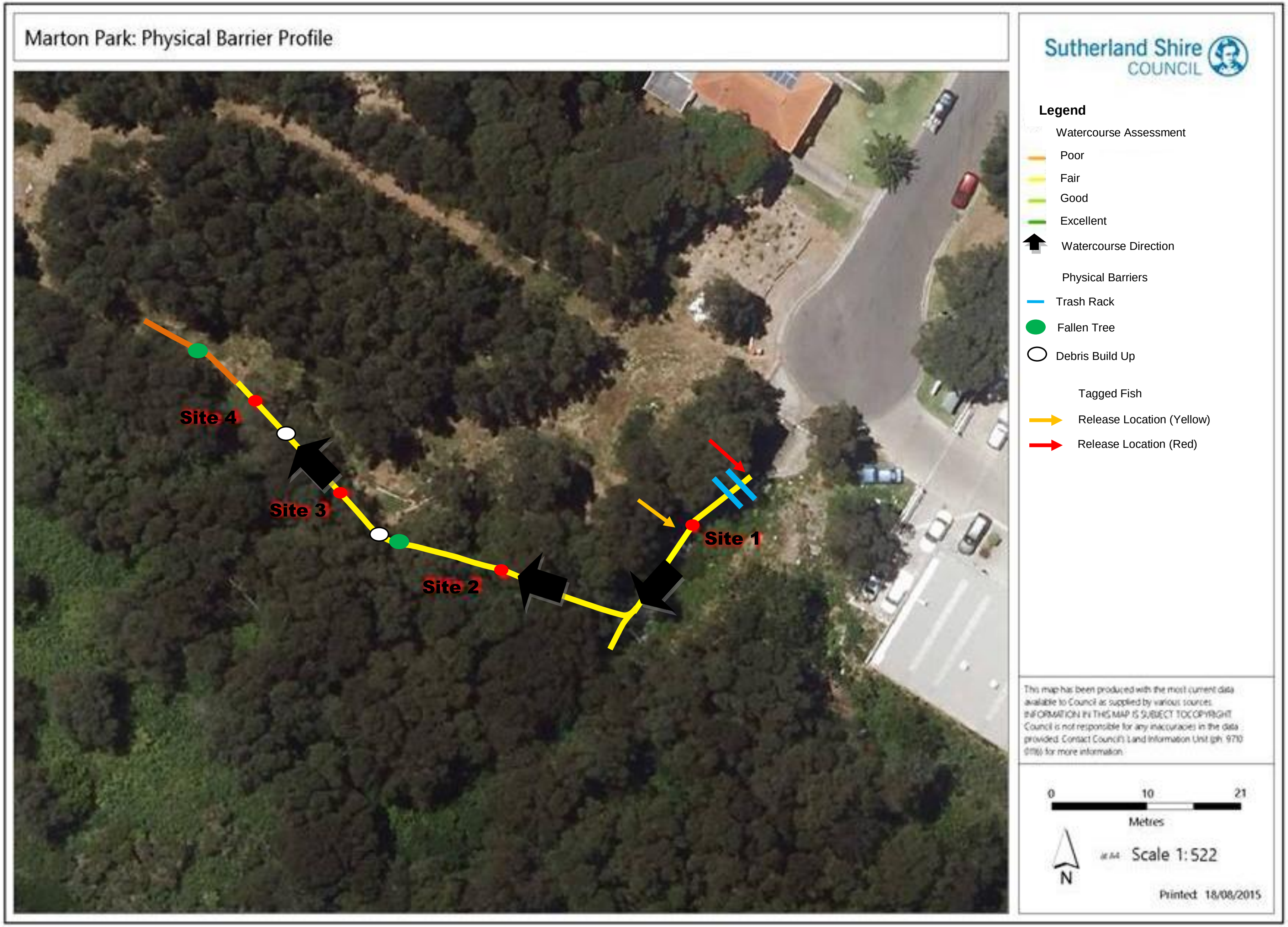

Plate 4.4 Physical barrier profile of Marton Park. Depicts major barriers that could potentially hinder the dispersal of G. holbrooki.

4.2 RE-ESTABLISHMENT OF *GAMBUSIA HOLBROOKI*

This investigation aimed to determine whether *Gambusia holbrooki* populations were able to re-establish in Carina Creek and Gwawley Creek, following their disappearance in flood waters. There has been no observation or indication that *Gambusia holbrooki* have re-established at Gwawley Creek or Carina Creek in the experimental time frame. The estimated time frame for which they have been observed to be absent is April 26 to October 7, 2015. Continuous monitoring and evaluation of these sites is still needed to be able to determine whether *G. holbrooki* are able to re-establish in the long term.

4.3 PHYSICO-CHEMICAL ANALYSIS

The range of physico-chemical parameters that *Gambusia holbrooki* were able to tolerate in this study was quite broad. At sites where *G. holbrooki* were present and abundant (Table 1), physico-chemical ranges were determined and compared against those locations that previously had established *G. holbrooki* populations, but were now lacking *G. holbrooki* (Table 2). Based on 9 weeks of physico-chemical data at Forbes Creek and Marton Park, this study has revealed that *G. holbrooki* were able to tolerate an overall pH range of 6.26-8.69, electrical conductivity of 314-1062 uS, turbidity of 0.1-15NTU, temperature of 9.6-15.9ºC, dissolved oxygen of 0.63-10.2ppm and vegetation cover of 0-95% (Table 2). In Gwawley Creek and Carina Creek where *G. holbrooki* are no longer established, the overall range in physico-chemical parameters observed were: pH of 6.01-8.17, electrical conductivity of 118-655uS, turbidity of 0.1-15.5 NTU, temperature of 10.3-17.9ºC, dissolved oxygen of 5.33-10.53ppm and vegetation cover of 0-65% (Table 2). It is apparent that the majority of these variables in Forbes Creek and Marton Park, and Carina Creek and Gwawley Creek, are very similar and overlap.

Table 4.2. Presence of Gambusia holbrooki in Marton Park and Forbes Creek. G. holbrooki is considered present in a site if there are consistent weekly occurrences of* G. holbrooki *of more than one individual. Relative abundance based on mean catch-per-unit effort over 9 weeks.

	Present	Absent
Forbes Creek		
Site 1	XXX	
Site 2a		0
Site 2b	X	
Site 3		0
Site 4	X	
Site 5		0
Site 6		0
Site 7		0
Site 8		0
Site 9		0
Marton Park		
Site 1	XX	
Site 2	XX	
Site 3	X	
Site 4	XX	

NB: Relative abundance - X=1-50 XX=50-100 XXX=100+

The environmental parameters that were measured during sampling did not vary significantly between the sites where *Gambusia holbrooki* were present (Forbes Creek and Marton Park) compared to where they were absent (Carina and Gwawley Creeks) (F=0.814; D.F=1,12; P(perm)=0.639) (Figure 4.10 and 4.11). However, there were significant differences in environmental variables between locations (F=13.301; D.F=2,12; P(perm)=0.001). Specifically there was a significant difference between Marton Park and Forbes Creek in terms of the environmental variables examined (t=4.54, P(perm)=0.031) (Figure 4.10 and 4.11). The main differences between these two locations were conductivity, dissolved oxygen concentrations, and turbidity; with Marton Park having higher averages in conductivity and turbidity, and considerably lower averages in dissolved oxygen concentrations. Physico-chemcial parameters were not significantly different between sites in Forbes Creek where *G. holbrooki* were present versus absent (F=2.15, DF=1,9, P(perm)=0.063) (Table 1) (Figure 4.12 and 4.13). However sites: 2b, 7 and 9 have considerable differences in conductivity, dissolved oxygen and vegetation cover respectively. A much higher conductivity level was recorded at Site 2b, a much lower concentration in dissolved oxygen was observed at site 7, and site 9 had a considerably lower percent vegetation cover than the other observed sites.

Table 4.2. Minimum and maximum values (range) of physico-chemical parameters at each location over the experimental time frame (9 weeks).Green locations/sites indicate where G. holbrooki are present. Blue locations/sites indicate where G. holbrooki are absent.

	pH	Conductivity (uS)	Turbidity (NTU)	Temperature* (°C)	Dissolved Oxygen (ppm)	Vegetation Cover (%)
Forbes Creek						
Site 1	7.09-8.66	314-715	3.5-4.3	10.5-14.1	8.62-10.2	0-75
Site 2b	7.01-8.44	730-1062	5.4-6.2	9.6-13.9	6.04-9.22	0-25
Site 4	7.22-8.69	321-638	1.5-4.2	9.8-13.6	9.44-10.05	0-90
Marton Park						
Site 1	6.26-6.48	365-928	0.8-10.5	13.7-15.9	2.58-7.6	5-90
Site 2	6.26-6.53	324-890	0.1-8.7	13.4-15.6	1.56-8.21	5-95
Site 3	6.34-7.01	332-913	0.1-15	13.3-14.8	0.83-6.95	5-75
Site 4	6.33-6.72	331-910	0.21-8	13.4-14.5	0.63-6.29	5-95
Gwawley Creek						
Site 1	6.01-6.7	118-655	0.4-15.5	12.1-17.9	5.33-9.48	0-5
Site 2	6.50-7.25	125-630	0.1-13.9	11.2-15.2	8.03-9.40	0-45
Site 3	6.63-7.19	158-615	0.1-12.5	11-15.1	7.55-9.25	0-30
Carina Creek						
Site 1	6.53-7.84	393-542	4-5.7	10.3-13.8	5.95-8.35	0
Site 2	6.78-8.16	378-450	3.2-5.6	10.6-13.6	8.25-9.02	0-65
Site 3	7.21-8.17	334-469	0.5-4.9	11.1-13.4	9.13-10.53	0-5

***Water temperature taken between hours of 9-11am**

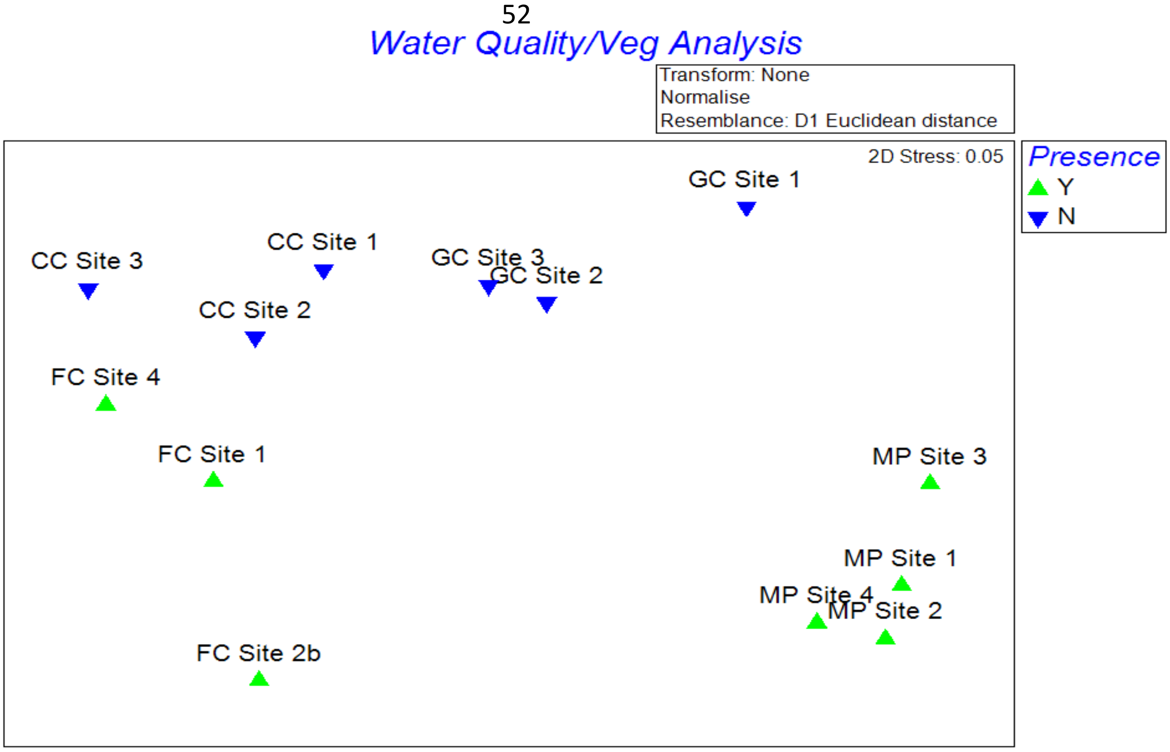

Figure 4.10. 2D MDS plot of environmental data with a Euclidian Distance resemblance matrix. Each point on the plot represents a sampling site. Environmental variables: pH, electrical conductivity (uS), turbidity (NTU), dissolved oxygen (ppm), temperature (°C) and mean vegetation cover (%).Green filled points represent sites within Forbes Creek (FC) and Marton Park (MP) where G. holbrooki is present. Blue filled points represent sites within Carina Creek (CC) and Gwawley Creek (GC) where G. holbrooki is absent.

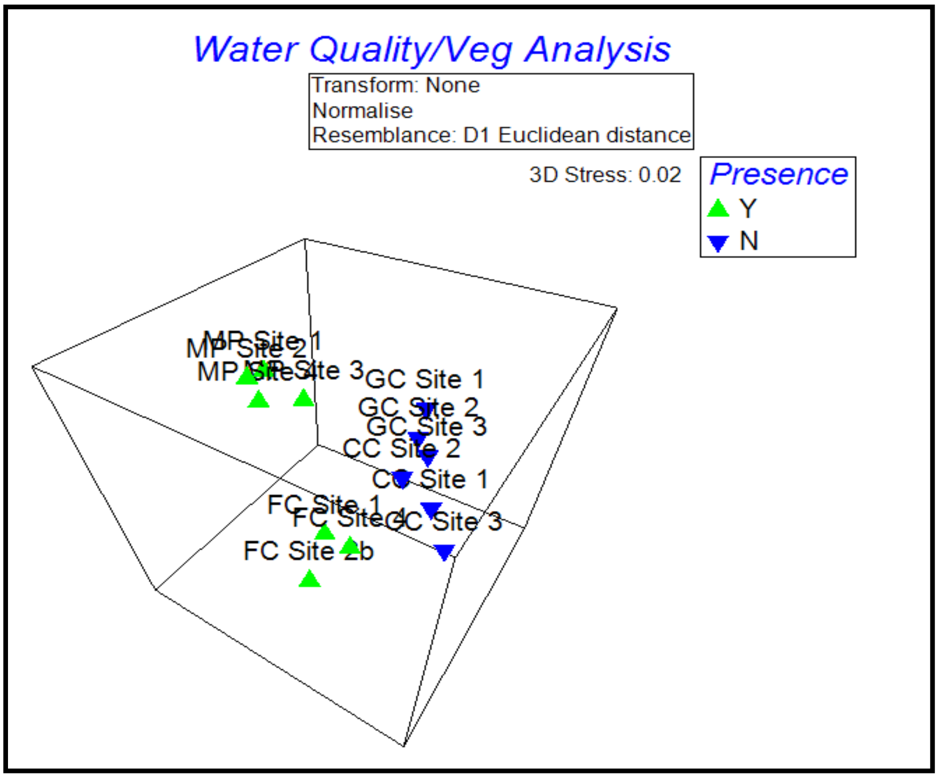

Figure 4.11 3D MDS plot of environmental data with Euclidean Distance resemblance matrix. Each point on the plot represents a sampling site. Green filled points represent sites within Forbes Creek (FC) and Marton Park (MP) where G. holbrooki is present. Blue filled points represent sites within Carina Creek (CC) and Gwawley Creek (GC) where G. holbrooki is absent.

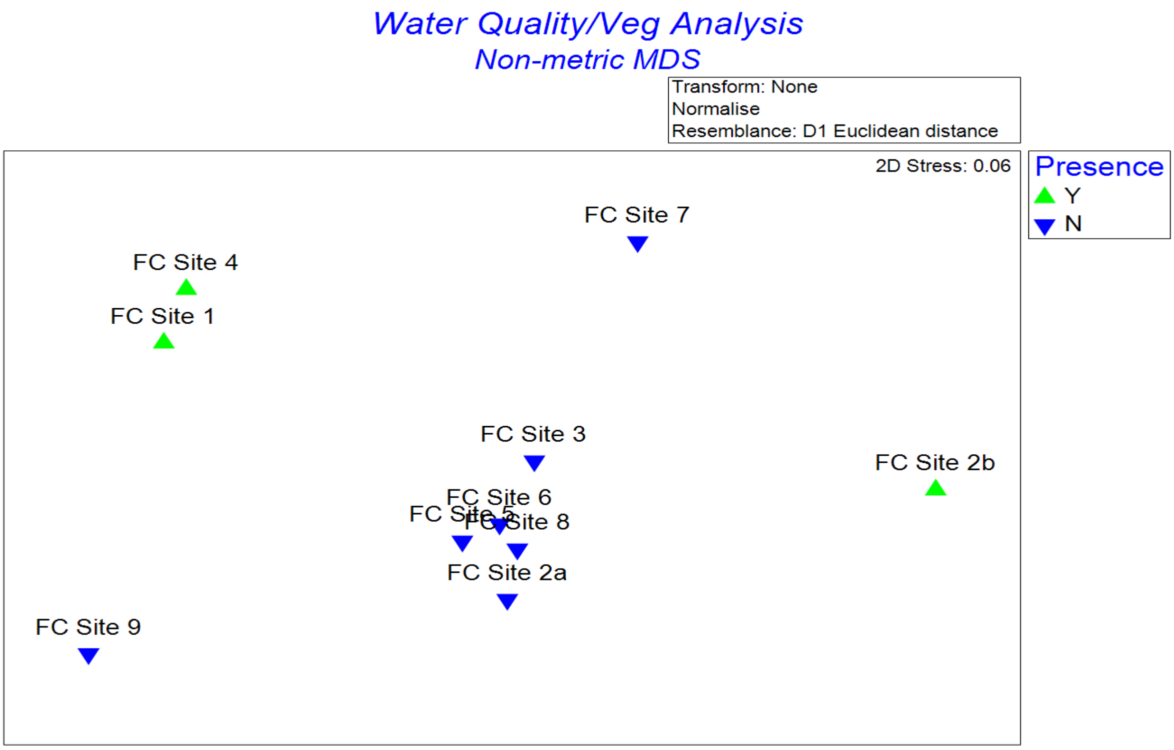

Figure 4.12 2D MDS plot of environmental data with a Euclidian Distance resemblance matrix. Each point on the plot represents a sampling site in Forbes Creek. Environmental variables: pH, electrical conductivity (uS), turbidity (NTU), dissolved oxygen (ppm), temperature (°C) and mean vegetation cover (%).Green filled points represent sites where* G. holbrooki *is present. Blue filled points represent sites where* G. holbrooki *is absent.

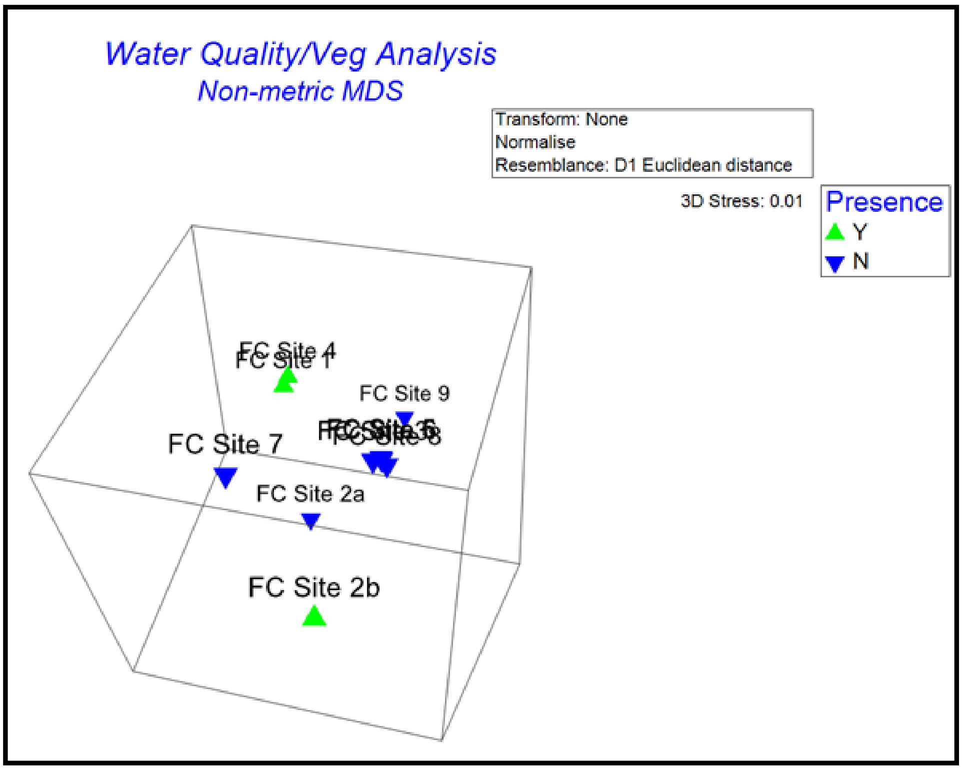

Figure 4.13 3D MDS plot of environmental data with Euclidean Distance resemblance matrix. Each point on the plot represents a sampling site at Forbes Creek. Green filled points represent sites where G. holbrooki is present. Blue filled points represent sites where G. holbrooki is absent.

4.4 RAINFALL PATTERNS AND TRENDS

During the experimental time period of June 2-August 11, 2015, the average amount of rainfall that was recorded in the area was 130mm (Figure 4.14). This is considerably lower than the average rainfall in the Sutherland Shire of 203mm in the last 10 years (Figure 4.14). These data are from four weather stations within the Sutherland Shire region (BOM, 2015).

Over the 9 weeks of sampling, rainfall was recorded and compared with the movement of re-captured individuals per week. There was no significant relationship between rainfall and distance travelled during these weeks at Marton Park and Forbes Creek (R^2=0.01, r=0.1, P=0.91) (Figure 4.15).

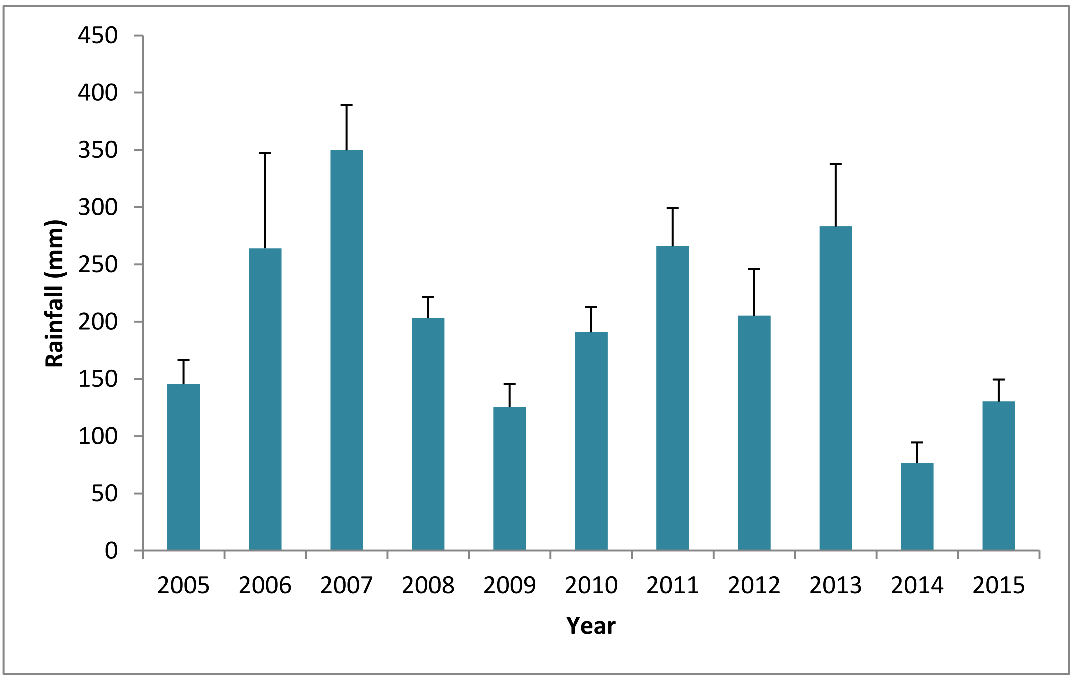

Figure 4.14 Average rainfall profile for 2005-2015. Based on rainfall data from June 2-August 11. Data was obtained and averaged from four weather stations in the Sutherland Shire region (Lucas Heights (ANSTO), Oyster Bay, San Souci and Audley (R.N.P)).Error bars are representative of the standard deviation of the four weather stations.

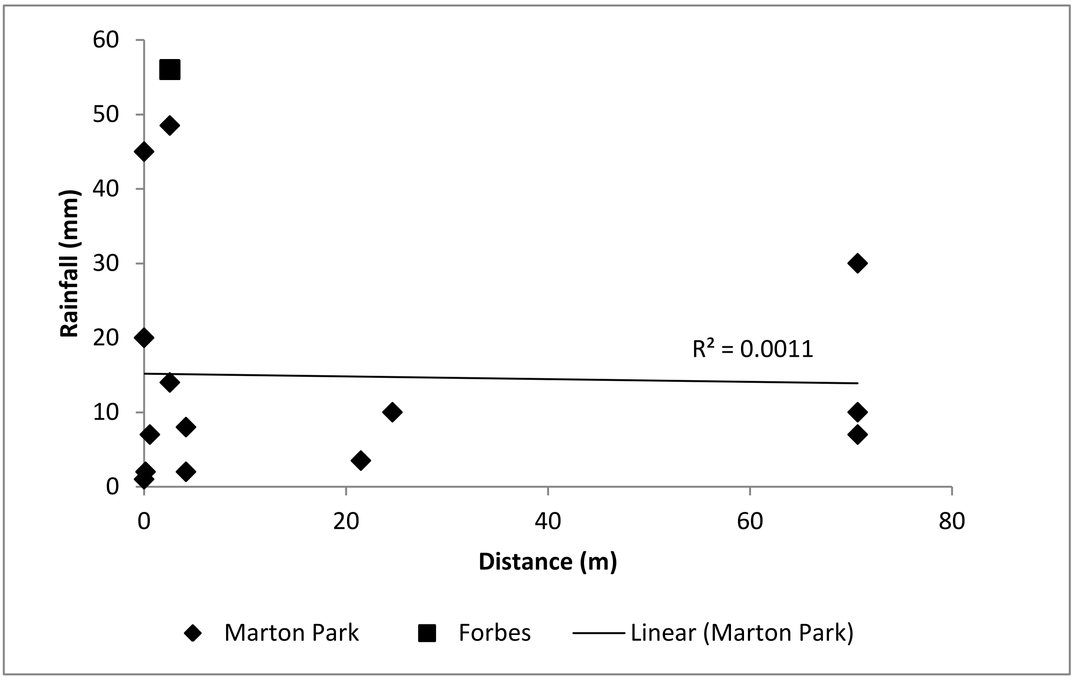

Figure 4.15 Distance travelled by **G. holbrooki** ***relative to rainfall at Marton Park and Forbes Creek. Each point represents an individual*** **G. holbrooki** ***that has travelled outside their release location. Forbes Creek is a single point that represents 14 tagged individuals and is not included in the analysis.***

5. DISCUSSION

5.1 PATTERNS OF RECAPTURE

The first goal of this study was to determine the movement and dispersal potential of *Gambusia holbrooki* within a freshwater system, over a given period of time. It was predicted that the frequency of recapture would be similar for both red and yellow populations (released upstream and downstream of the trash rack respectively), at each location, but their dispersal would vary based on the number and types of barriers present. However, results show that there is a considerable difference in the frequency of recapture below versus above the trash rack (yellow-tagged versus red-tagged individuals) between Forbes Creek and Marton Park (Figure 4.1). From this, the study shows that dispersal and individual movement of *G. holbrooki* is highly variable and depends greatly on site specific factors and stream connectivity, which is evidenced to have a major influence on the patterns of dispersal of this invasive fish species (Murphy *et al*, 2015).

It is suggested that at Forbes Creek, red-tagged individuals were re-captured more often due to the smaller geographic boundary that they were observed in compared to the yellow-tagged individuals. Therefore the probability of re-capturing red-tagged individuals was greater due to the limited dispersal of red-tagged fish observed at this site compared to the yellow-tagged individuals, which had dispersed outside of their initial release site and thus spanned a larger geographic area. A considerable proportion of the yellow-tagged individuals may have also dispersed downstream to areas of Forbes Creek that were physically inaccessible for sampling, and therefore were unable to be re-captured, and thus lost from the sampling pool (Pope *et al,* 2010). At Marton Park, re-capturing was unable to be conducted upstream of the trash rack where the red-tagged individuals were initially released. This is due to the persistent presence of petrochemicals concentrated in this zone of the wetland, in addition to this zone being fenced off and therefore largely inaccessible to conduct sampling. Therefore, a lower frequency of red-tagged individuals could be at least partly explained by the inability to conduct sampling in the zone where they were initially released; thus incidences of re-capture of red-tagged individuals were only possible if they dispersed downstream of the trash rack.

In this study, yellow-tagged individuals at Forbes Creek were not observed or re-captured following their initial release until five weeks after their initial release. During these five weeks, it is suggested that these tagged individuals had settled in areas within Forbes Creek that were inaccessible to sample - or areas that were not sampled due to high safety risk - and then subsequently dispersed into areas where sampling was able to be safely conducted. Other possible explanations are suggested to be influenced by improvements in the precision and accuracy of the netting technique over time (Pope *et al,* 2010). The ability to capture these individuals is mostly attributed to the skill and technique of the netter, especially if there are considerably smaller populations of tagged individuals to capture as a result of high dispersal and increased mortality (Thompson, 2003). It is for this reason that both field based experimentation *and* manipulative lab studies should be conducted, especially in determining *which* physical and chemical variables are the most effective in inhibiting and promoting the dispersal potential of *Gambusia holbrooki* populations. Manipulative lab experimentation provides the added benefit of controlling and manipulating variables that cannot be manipulated, and are largely unpredictable, in field experimentation.

The correlation between body size, sex and distance travelled by *Gambusia holbrooki* was deemed negligible in this study. Past work suggests that variation in dispersal is also due to the variation in underlying behavioural traits in individual fish, in addition to the influence of their physical and chemical surroundings (Rehage and Sih, 2004). It is this individual variation in dispersal behaviour and in behavioural traits - such as ‘boldness’ to explore surrounding areas and pursue the most suitable habitat - that could contribute considerably to the patterns of dispersal seen in this study (Bradford and Taylor, 1997; Fraser *et al,* 2001;Rehage and Sih, 2004). Food-dependent and density dependent triggers are also seen to influence the dispersal behaviour of *G. holbrooki,* preferring areas where food and shelter resources are abundant (Bengtsson *et* al, 1994). By assessing these other influencing factors as potential impacts on the dispersal patterns of *G. holbrooki*, it can help us further understand the distribution of *G. holbrooki* within Marton Park Wetland and Forbes Creek in the Sutherland Shire.

5.2 RECAPTURE AND PHYSICAL BARRIERS

In an effort to distinguish which physical barriers are the most effective at inhibiting and promoting the movement of *Gambusia holbrooki*, this study focused on determining how certain natural and anthropogenic barriers influence the patterns of dispersal observed at Forbes Creek and Marton Park Wetland. It was predicted that the same type of barriers would yield similar dispersal results regardless of the location. However, the results of this study suggest that the dispersal potential of *G. holbrooki* is very site specific and is thus found to be highly variable between locations even if locations share the same types of physical barriers. This is due to the variation in the types of physical barriers present, the nature of these barriers, their location in the freshwater system and their ability to influence stream connectivity (Fullerton *et al,* 2010). In terms of the distribution of both red and yellow-tagged individuals within both of the locations, it is found that in Forbes Creek *all* of the observed red-tagged individuals were re-captured at their initial release site, whereas *all* of the observed yellow-tagged individuals were re-captured *outside* of their initial release location. In Marton Park, the opposite was true, *all* of the observed red-tagged individuals were re-captured outside their initial release site, and *all* yellow tagged individuals were re-captured within their site of release. This result is evidence in itself that there are many site specific influences, as well as behavioural influences that could contribute to the explanation of their dispersal pattern in this study.

5.2.1 NATURAL BARRIERS

In regard to the natural barriers at Forbes Creek and Marton Park, there is variation in the effectiveness of these barriers such as fallen trees, debris build-up and landscape formations such as knickpoints at hindering the downstream dispersal of *Gambusia holbrooki*, due to the varying degrees to which they can influence stream connectivity. In the context of natural physical barriers, stream connectivity is dependent on the characteristics of each physical barrier and the formation of the landscape around this barrier (Faulks *et al,* 2010). Therefore, the effectiveness of these barriers to promote and hinder dispersal of stream fishes also varies.

At Forbes Creek, it has been observed that knickpoints at this site have not shown to hinder the movement of *Gambusia holbrooki* downstream, but could actually have encouraged their movement as a result of increases in stream velocity associated with increases in channel gradient. There are three main possibilities that explain the movement of all observed yellow-tagged individuals to only one observed site location within Forbes Creek outside their initial release site. These include: (1) the lack of sufficient time for *G. holbrooki* to continue to disperse further downstream, (2) the large build up of debris extending the entire creek width has inhibited their movement further downstream and (3) the abiotic conditions (at Site 4), such as deeper water, are optimal for their survival in winter months and thus the behavioural trait to pursue a more suitable habitat is not required. Contrastingly at Marton Park, dispersal patterns of tagged individuals have shown that natural barriers such as fallen trees and debris build-up have not hindered their movement downstream, so it may be unlikely that the build-up of debris downstream of Forbes Creek is the variable hindering their continual dispersal.

Interestingly at Marton Park, the lack of observed movement of yellow-tagged individuals outside of their initial site of release was evident, even though red-tagged individuals were observed to have moved much further downstream from their release site. If it is possible for the red-tagged individuals to have dispersed a considerable distance downstream through the physical barriers present, there is no obvious reason as to why yellow-tagged individuals would not be able to do the same. There was no significant physical barrier hindering their movement downstream, and there is speculation as to why there was no observed movement of the yellow-tagged individuals. It is likely that other underlying characteristics are influencing this lack of observed movement downstream, such as their presence in areas of Marton Park which were inaccessible to sample, due to safety risk and presence of dense bush on the banks. The inaccessibility to several pockets of the wetland was the main limitation in determining very accurate dispersal patterns in this location.

There was no significant observation or evidence showing that the natural barriers focused on in this study exclusively hinder, or exclusively promote, *Gambusia holbrooki* dispersal further downstream. However the importance of landscape structure and formation, and the variation in environmental variables within the landscape is evidenced as an important factor for stream connectivity, and in extension fish passage through freshwater environments (Faulks *et al*, 2012; Murphy, 2015). Karim *et al* (2012) suggests in their study of hydrological connectivity in a wetland environment similar to Marton Park, that riverbank elevation, land relief and height of levee banks are the main influencing factors that control the degree of stream connectivity and therefore fish passage within and between freshwater environments, such as between rivers and wetlands; especially during flooding events. In Marton Park its low elevation makes it prone to flooding during major storm events, and thus there is the capability for smaller freshwater environments nearby to become infiltrated with *G. holbrooki* during flooding events as a result of hydrological connectivity that may occur between the wetland and other freshwater bodies during flooding (Chapman and Warburton, 2006). In understanding how connectivity plays a role in freshwater fish dispersal and ecology, addressing river structure, elevation, temporal complexity and quantifying the force and direction of flow are among important factors to be considered, in addition to any influential physical barriers evident (Fullerton *et al*, 2010). To determine network connectivity and dispersal barriers at a regional scale, the use of GIS tools may predict larger scale factors which influence local factors and vice versa (Spens *et al*, 2007).

5.2.2 ANTHROPOGENIC BARRIERS

The main anthropogenic barrier considered in this study is the presence of trash-racks within a freshwater system. These physical barriers are designed to prevent large volumes of trash from moving further downstream and are used to manage pollution by accumulating trash in urban creeks and other freshwater systems with considerable human activity occurring in the surrounding area. Results emphasise that the trash rack present at Forbes Creek is highly effective at hindering the dispersal of all observed red-tagged individuals at this location, for which no observed red-tags moved through this barrier. Conversely, at Marton Park there was no evidence to suggest that the trash racks found at this site played any considerable role in hindering their dispersal, and red-tagged individuals were observed to have dispersed further downstream of this barrier.

Anthropogenic physical barriers may act to either encourage or exclude fish passage depending on the architecture and design of the barrier, the purpose of the barrier, and its dimensions (Faulks *et al*, 2010). Due to the variation of these elements, trash racks can vary considerably between sites depending also on the estimated trash load and dimensions of the creek. Not all trash racks are the same, and differences in their size, bar spacing, bar shape and rack angle have shown to influence the dispersal of fish species and other aquatic animals in freshwater systems (Floyd, 2007; Raynal *et al*, 2014). At all four locations in this study, all trash racks had the same 90 degree rack angle (vertical), bar shape (cylindrical) and bar spacing, but differed in size and amount of trash accumulated (See Figures 3.1 to 3.4). Therefore it is suggested that only differences in size of the trash rack and variations in trash accumulation could have possibly influenced dispersal in this study. The width and length of these trash racks are also observed to be relative to the size and dimensions of the creeks. It would be valuable to the findings in this study to undertake research in the future that experimentally examines how the above variables in trash rack design and trash accumulation affect fish movement.

At Forbes Creek, it has been observed that the trash rack is effective at obstructing the movement of *Gambusia holbrooki* downstream, with all of the observed red-tagged individuals not dispersing from their source site. It should be noted however, that the trash rack at this location had not been cleared of accumulated trash for the duration of the study, and there was a significant accumulation of trash and leaf litter obstructing the bar spaces of the trash rack. It is suggested that the uncleared state of the trash rack was effective at hindering their movement at this location, and that the clearing out the trash rack may have resulted in different findings. This has been observed to act as an effective barrier to limit the dispersal of *G. holbrooki* at this location, providing that it is not cleared of trash. Contrastingly, at Marton Park the dispersal patterns of tagged individuals have shown that trash racks have not hindered their movement downstream. However, the trash rack at this location was completely cleared of rubbish, which obstructed the bar spaces, explaining the movement of *G. holbrooki* downstream at this site as opposed to Forbes Creek. The state of trash accumulation is therefore an important variable in this study in understanding *G. holbrooki*'s dispersal potential in freshwater systems with trash racks present. A potential management strategy to aid the exclusion of *G. holbrooki* into other connecting freshwater habitats would be to only partially clear these trash racks of garbage, or install mesh barriers on these trash racks. However, the consequence of this would be that it would also inhibit the dispersal of other native fish species and other aquatic wildlife (Fullerton *et al* 2010; NSW DPI, 2015).

Past studies on fish passage and movement through trash-racks indicate that the most important variables in the architecture and implementation of these barriers include: (1) bar spacing (2) water velocity approaching the trash-rack, (3) angle of inclination of the trash rack, (4) debris accumulation and (5) bar shape (Floyd *et al*, 2007; Hanson and Li, 1983; Raynal *et al*, 2013; Raynal *et al,* 2014). Early studies by Hanson and Li (1983) found that bar spacing and differences in bar spacing between trash racks had a significant effect on the behavioural response of Chinook salmon and American shad, with the bar spaces ranging from 5.1cm to 30.5cm. It was found that passage through trash-racks decreased significantly through bar spacing less than 22cm, for juveniles of 35-75mm in size. These researchers also found a correlation between water velocity and movement through trash racks, with passage significantly reduced at a water velocity of 30.5 cm/s or less. However, these findings are specific to the species of fish in the study, and do not reflect the dispersal potential of *Gambusia holbrooki* under the same conditions. Further lab study and field experimentation on the effects of bar spacing and water velocity on the dispersal potential of *G. holbrooki* will further strengthen the results found in my study. The bar spacing of all locations studied in the Sutherland Shire were the same, with a bar spacing of 10cm.

Later research by Raynal *et al* (2013/14) revealed that the shape of the bar and the screen inclination of the rack have an influence on fish passage. More specifically, it was suggested that the inclination of the trash rack also influenced the water velocity approaching the trash rack, which in turn affects the rate of fish passage through the barrier. New research specific to *Gambusia holbrooki* by Murphy *et al,* (2015) contradicts this by suggesting that for mosquitofish specifically, anthropogenic impacts and barriers appear to be less important than natural abiotic factors in regulating mosquitofish presence. However, this study was limited to the Iberian Peninsula, and therefore not all anthropocentric activities and barriers are represented. My study does not provide information regarding the effects of anthropogenic activity compared to the effects natural, abiotic factors. However, further research exploring the extent to which anthropogenic and industrial activity affects the pollution and oxygen concentrations in freshwater creeks, and the effect of this on vertebrate, macro-invertebrate, and vegetation structure in these creeks, would help to strengthen my findings and contribute to the further understanding of *G. holbrooki* tolerances in the Sutherland Shire.

5.3 RE-ESTABLISHMENT OF GAMBUSIA HOLBROOKI

It is critical to determine the combination of variables and factors that influence ecosystem invisibility, as this provides important information on biological invasion and influences decisions regarding management and conservation (Maceda-Veiga *et al,* 2013). It was predicted that *Gambusia holbrooki* would be able to re-establish in the short-term in sites they previously inhabited after flooding, given their incredible aptitude to establish in a variety of environmental conditions with incredible invasive success. However, results of this study show that natural re-establishment has not occurred in the short-term at Gwawley Creek and Carina Creek. However it is still important to consider that *G. holbrooki* may still re-establish in the long term, and therefore continuous monitoring and evaluating is still needed to ensure that these freshwater habitats remain free from this invasive pest.

Factors that affect the invisibility of an alien species can be both environmental and anthropogenic in nature. Recent research has found that the major contributing factors in hindering the re-establishment of invasive species include: (1) pollution and eutrophication via anthropogenic activity and, (2) geological and hydrological features present in the landscape (Kesler, *et al,* 2010; Maceda-Veiga *et al,* 2013; Novo *et al,* 2015). However, a recent study by Midgley *et al* (2014) has found no significant difference in the density of *Gambusia holbrooki* among water sources of various pollutant concentrations, emphasising their exceedingly high tolerance to anthropogenic pollutants. This is also supported by my study at Marton Park, where high densities of *G. holbrooki* persist in the presence of petrochemical pollution. This indicates that it is unlikely that pollution is the factor influencing their re-establishment at the creek sites in this study, as well as the lack of obvious chemical pollution found at these sites. Additionally, there were no significant geological features such as knickpoints, or hydrological features such as a reservoirs, spillways, or weirs (Buysse *et al,* 2008), present at either site that would have significantly hindered their re-establishment. However, both of these creeks feed directly into Carina Bay and Gwawley Bay, which have much higher salinity levels than Carina Creek and Gwawley Creek respectively, and a large portion of the population could have perished as a result of this. Still, this is merely speculative and could also be due to the insufficient time for *G. holbrooki* to disperse or the inability to disperse upstream back into the creek sites.

Other influential factors that may be affecting the short-term re-establishment of *Gambusia holbrooki* include the lack of connectivity between locations with and without *G. holbrooki.* Connectivity can be established through natural barriers and events such as flooding, or anthropogenic activities and barriers such as construction of temporary or permanent watercourse diversions (DNRM, 2014). Ecological influences such as the mode of reproduction of these fish, the potential for juveniles to disperse, and other behavioural dispersal mechanisms are also shown to be influential factors in recovery after local extinction and re-establishment of both native and invasive species (Knapp and Sarnelle, 2008). My study is limited in that it does not provide information regarding the potential mechanisms influencing the re-establishment and dispersal of *G. holbrooki* at these sites, only the patterns. Accidental human-assisted dispersal is still a major influence in the distribution of *G. holbrooki* in Australia due to the misidentification of *G. holbrooki* by the general public as a native species (Linterman, 2004). In order to ensure that *G. holbrooki* remains absent from Gwawley Creek and Carina Creek in the long-term, educational signposting, awareness campaigns and continual monitoring of these sites is needed.

5.4 RECAPTURE AND PHYSICO-CHEMICAL BARRIERS

Another main goal of this study was to determine whether there were any significant differences in the physico-chemical environment where *Gambusia holbrooki* were present, compared to where they were absent. This would give us insight into which parameters are more influential in dictating *G. holbrooki* presence than others. It was predicted that vegetation density and water flow regime would be the major determinants of whether *G. holbrooki* would be present or absent at a site. This study found that there was no considerable difference in the ranges of the parameters observed in the locations where they were present compared to where they were absent (Table 2) (Appendix 8 and 9). Based on the physico-chemical parameters studied, we can infer that conditions are appropriate at

Carina Creek and Gwawley Creek (where they were not observed) for *G. holbrooki* to re-establish once more. However, not *all* physico-chemical parameters could be analysed in this study, and additional variables that could have strengthened this analysis include flow rate of the water (m/s) and further chemical analyses of pollution concentrations, which would provide further insight to this study. It should also be emphasised that the results in this study were largely collected in the winter months where the water temperature was lower, and a focus on dispersal in summer may yield different outcomes.

My work did suggest that there was a significant difference in conductivity, dissolved oxygen and turbidity between Forbes Creek and Marton Park. However, this is not particularly insightful, as both of these locations already have persisting *Gambusia holbrooki* populations. Therefore commenting on how the differences in parameters between these locations in relation to the presence and absence of *G. holbrooki* would be futile. This further highlights the species ability to tolerate a very wide range of conditions, and be able to not only survive, but have reproductive success in a wide range of environmental conditions and habitats (NPWS, 2003; Pyke, 2008).

In contrast, findings at Forbes Creek confirm that just two out of nine sites that have been able to sustain *G. holbrooki* populations. It is suggested that vegetation cover is a very significant influence in this study. Sites 1 and 4 in Forbes Creek where *G. holbrooki* are present have considerably higher vegetation cover than all the other sites and appears to be a very influential factor in their establishment (Appendix 9). It has been highlighted in previous studies, that the presence of at least a moderate amount of bank and macrophyte vegetation is preferred by *G. holbrooki* for their persistence in a freshwater habitat (Lloyd *et al*, 1986; Lund, 1999 and Webb *et al*, 2007). This supports the predictions that this study had that the presence and density of vegetation is a highly influential factor in determining potential sites for *G. holbrooki* to establish and thrive. My work also suggests that flow rate could influence the presence of *G. holbrooki*. This is supported by early work by Cadwallader and Backhouse (1983) and Meffe and Snelson, (1989), which highlights that *G. holbrooki* are only ever found in areas that have low discharge and flow rates. However, further research is needed to determine the relationship between flow rate and *G. holbrooki* presence, as well as the tolerance of *G. holbrooki* to varying flow rate levels, at the sites studied in the Sutherland Shire.

The petrochemical pollution observed at Marton Park was not considered an influential chemical barrier that hindered the ability for *G. holbrooki* to survive in the short term in this study. However, long term effects of pollutants could result in the alteration of *G. holbrooki* population dynamics, including changes in: phenotypic sex ratio, structure, size and biomass of the population (Midgley *et al*, 2014). Increases in the concentration of the pollution may also act to compromise the ability for *G. holbrooki* to persist and survive at high densities, as a result of significant oxygen depletion caused by excessive pollution (Kesler *et al,* 2010). Additionally, it hinders the potential establishment of native fish species in this part of the wetland. The presence of petrochemical pollution also has negative consequences for the frog populations present at this location. Marton Park and Forbes Creek were the only sites where there was evidence of frog populations. At Marton Park, the Striped Marsh Frog (*Limnodynastes peronii*) was the only species of frog observed at the tadpole life stage. At Forbes Creek, the Striped Marsh Frog and the Common Eastern Froglet (*Crinia signifera*) were the species identified at the tadpole life stage. No other fish species were observed at all in any of the sites in this study.

5.5 RAINFALL AND DISPERSAL PATTERNS

There is no evident trend in this study showing that rainfall results in an increase in the distance dispersed by *G. holbrooki* even though it was predicted that higher rainfall would lead to greater dispersal. Past studies that look at the dispersal of fish via flooding show that there must be an extreme rainfall event nearer to flood proportions for there to be a significant effect on the dispersal patterns of many fish (Chapman and Kramer, 1991; Chapman and Warburton, 2006; Labbe and Fausch, 2000). Whether the severity of the flood event and distance dispersed by the fish is proportional to body size is yet to be determined in the literature. However even during extreme flooding, other factors such as the slope of the land, stream volume and flow rate all influence the dispersal potential of fish, and therefore these variables also need to be considered in addition to the amount of rain that has fallen (Chapman and Kramer, 1991).

Over the experimental time frame of this study, the rainfall average of 130mm was well below the average of 203mm from the last 10 years. The considerable difference in rainfall from the average is suggested to be the primary reason for the lack of movement observed by *G. holbrooki* in this study. The extreme storm event at the end of April, 2015 saw more than 100mm of rain fall in just one day. This resulted in the disappearance of *G. holbrooki* from two of my study locations. The highest amount of rainfall recorded within the study period was 46mm on the 19th June (BOM, 2015). It is therefore suggested that only rainfall events of more than 50mm of rain per day would have any considerable influence on the dispersal of *G. holbrooki*. However, this is an estimate based on observations in this study, and further research needs to be conducted to be able to determine this number more accurately.

5.6 DIRECTIONS FOR FUTURE STUDIES

The findings and results of this research highlight further knowledge gaps that need to be addressed regarding the mechanisms of dispersal of *Gambusia holbrooki;* and more specifically the non-behavioural dispersal mechanisms underlying their movement within the Sutherland Shire landscape. With regards to *Gambusia* and other invasive fish species, many studies have investigated dispersal behaviour and genetics and its link to invasiveness and dispersal potential (Alemadi and Jenkins, 2008; Brown, 1985; Cote *et al,* 2010; Rehage and Sih, 2004), yet no studies on the effects of landscape on *G. holbrooki* dispersal have been conducted. By being able to determine the potential for *G. holbrooki* to disperse in habitats and environments with varying geological and hydrological complexity, will give better estimations for conservationists about which environments *G. holbrooki* are able to disperse the most efficiently in. This will allow conservation efforts to be concentrated in areas where their dispersal potential is much greater, and be able to hinder their movement into uninvaded freshwater systems. Additionally, further studies on other non-behavioural dispersal mechanisms such as dispersal via the transportation of juvenile *G. holbrooki* by birds would be appropriate to be able to determine whether this is an additional contributor to their pervasive presence in NSW.

Further research also needs to be directed towards lab-based manipulative experimentation, to determine which physical barriers prove most effective at hampering the dispersal of *Gambusia holbrooki*. My research has looked at the dispersal potential of *G. holbrooki* in relation to various physical barriers already present in creeks in the Sutherland Shire. However, further studies assessing the effectiveness of other anthropogenic and natural barriers would further strengthen this research, especially by incorporating a manipulative lab-based experimental approach. This would also complement the numerous studies on population connectivity of stream fishes and the link between stream connectivity and invasiveness (Adams *et al,* 2001; Crooks and Suarez, 2006; Faulks *et al,* 2010; Rahel, 2007). Additional field experimentation at the same locations in the Sutherland Shire should also be conducted in regarding the relationship between vegetation density and *G. holbrooki* presence. The manipulation of vegetation density at these locations using artificial macrophytes can further test the importance of vegetation density as a variable that influences *G. holbrooki* presence, and can be compared with this study to see if there are any significantly different results. Additionally, this project has been conducted in the winter months where the densities of G.holbrooki populations are at their lowest (NPWS, 2003). A similar study should be done in their breeding season in the summer months to be compared with the results found in this study. This could further highlight the role of density-dependent dispersal in this species and may yield some interesting results as a result of seasonal changes. For the safeguarding of native species and ecological communities from *G. holbrooki* invasions, it is imperative that the dispersal potential of *G. holbrooki* is researched in more significant detail, not only on a genetic and behavioural level, but in relation to landscape structure, barrier type and changes in micro-climate and season.

6. CONCLUSIONS

This study has highlighted how the dispersal potential of *Gambusia holbrooki* through a freshwater system can be affected by physical and chemical barriers, even in a species with an incredible aptitude for invading and establishing in a variety of freshwater environments. This study has also attempted to determine whether *G. holbrooki* have the ability to re-establish at specific sites within the Sutherland Shire that they previously inhabited in the short term. This is, however, one of the first attempts to assess the dispersal ability of *G. holbrooki* in the presence of *both* natural and anthropogenic physical barriers, as well as more pervasive chemical barriers such as the potential to persist in a polluted environment in the Sutherland Shire. This study will hopefully provide a springboard for a range of future studies that can use this model species to further establish which physical and chemical barriers are the most effective at hindering this species from establishing and re-establishing in freshwater systems in the Sutherland Shire. The Sutherland Shire and the Royal National Park are represented by some of the most important freshwater ecosystems and ecological communities, which are in danger of experiencing ecological damage associated with an invasive species such as loss of biodiversity of natives. The dispersal ability and potential of *G. holbrooki* in a variety of freshwater environments need to be well understood if there is any chance of successful mitigation and future for diverse freshwater habitats at a local and regional scale.

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Appendix 1: NSW River Survey records of *Gambusia*

Study catchments and their rivers where *Gambusia* populations were recorded during the NSW Rivers Survey 1994-1996 (Harris and Gehrke, 1997)

<u>Clarence River Catchment</u>

Rivers: Clarence, O'Rara

<u>Gwydir River Catchment</u>

Rivers: Horton, Gwydir

<u>Hawkesbury River Catchment</u>

Rivers: Cox's, Mangrove Creek

<u>Hunter River Catchment</u>

Rivers: Hunter, Goulburn

<u>Lachlan River Catchment</u>

River: Retreat

<u>Macleay River Catchment,</u>

Rivers: Gara, Macleay

<u>Macquarie River Catchment</u>

Rivers: Bogan, Duckmaloi, Fish, Turon, Little, Talbragar Macquarie

<u>Manning River Catchment</u>

River: Gloucester

<u>Murrumbidgee River Catchment</u>

Rivers: Yass, Colombo Creek

<u>Namoi River Catchment</u>

Rivers: MacDonald, Peel, Cockburn

<u>Richmond River Catchment</u>

Rivers: Richmond, Leycester Creek

<u>Shoalhaven River Catchment</u>

River: Shoalhaven

Appendix 2: Forbes Creek Site Photos

Figure 2a) Site 1: Upstream from trash rack

Figure 2b) Site 2a: Downstream from trash rack

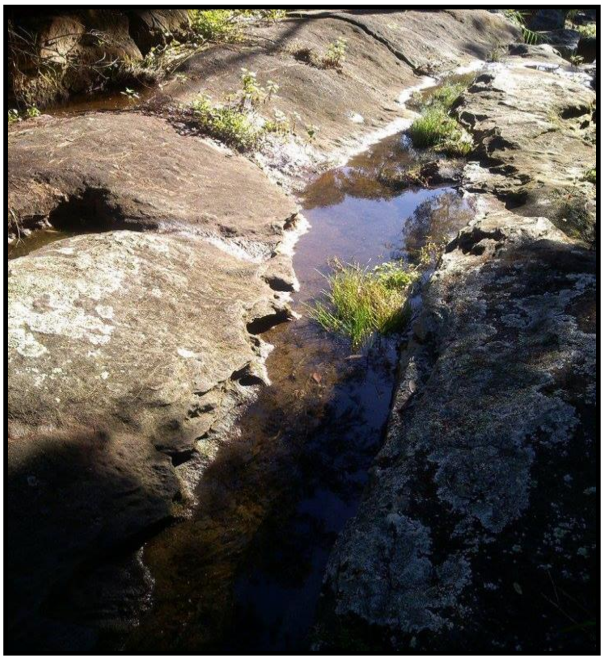

Figure 2c) Site 2b: Downstream from trash rack parallel to site 2a.

Figure 2d) Site 3: Downstream from Site 2.

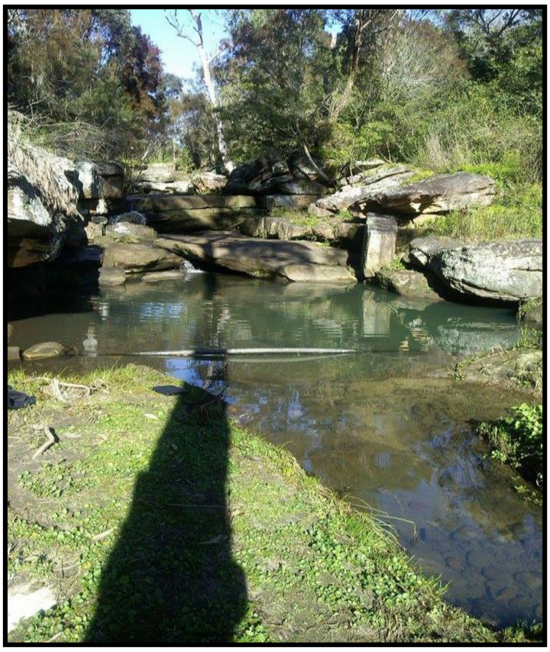

Figure 2e) Site 4: Downstream of Site 3.

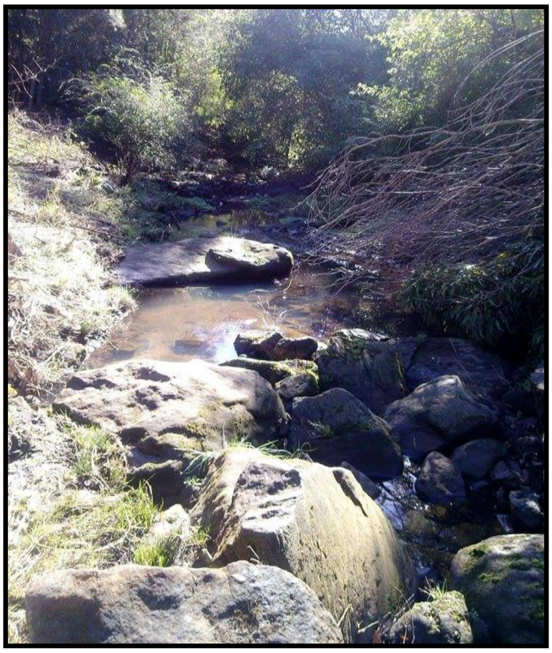

Figure 2f) Site 5: Downstream of Site 4.

Figure 2g) Site 6: Downstream of Site 5.

Figure 2h) Site 7: Downstream of Site 6

Figure 2i) Site 8: Upstream of Site 1.

Figure 2j) Site 9: Upstream of Site 8.

Appendix 3: Marton Park Wetland Site Photos

Figure 3a) Site 1: Downstream from trash rack (in picture).

Figure 3b) Site 2: Downstream from Site 1.

Figure 3c) Site 3: Downstream from Site 2.

Figure 3d) Site 4: Downstream from Site 3.

Appendix 4: Gwawley Creek Site Photos

Figure 4a) Site 1: Upstream from trash rack.

Figure 4b) Site 2: Downstream from trash rack.

Figure 4c) Site 3: Downstream from Site 2.

Appendix 5: Carina Creek Site Photos

Figure 5a) Site 1: Upstream from trash rack (shown).

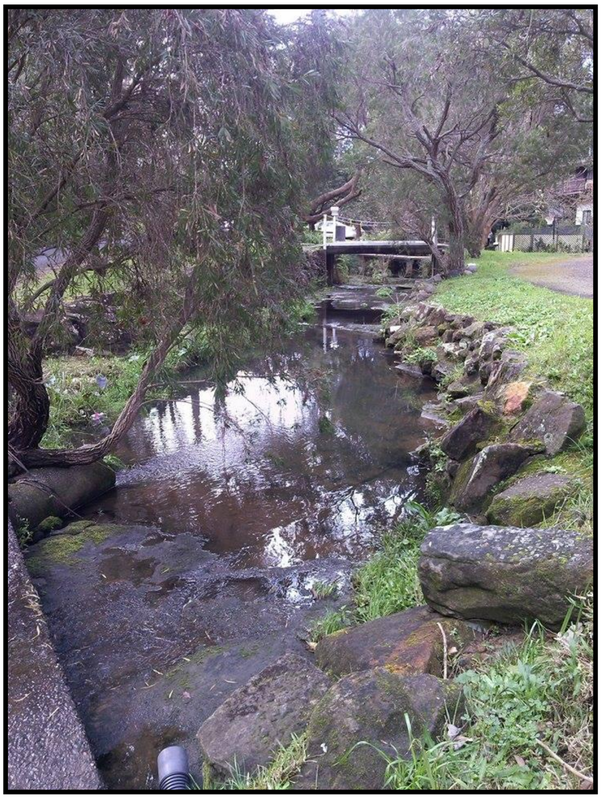

Figure 5b) Site 2: Downstream from Site 1.

Figure 5c) Site 3: Downstream from Site 2.

Appendix 6: Sampling Site Dimensions of Forbes Creek and Marton Park Wetland

Table 1. Site Dimensions for Forbes Creek and Marton Park Sampling Sites.

Site Dimensions	Length of Site (m)	Width at Widest Point (m)	Width at Narrowest Point (m)
Forbes Creek			
Site 1	12	8.2	5.5
Site 2a	20	5.5	1.6
Site 2b	14.4	0.8	0.3
Site 3	24	4.2	0.8
Site 4	16.5	6	0.5
Site 5	10.1	2.4	1.6
Site 6	22.6	3.7	2.5
Site 7	25	1.7	0.3
Site 8	26.4	3	0.9
Site 9	21.4	7.2	1.3
Marton Park			
Site 1	21.5	5	4.5
Site 2	21	4	0.8
Site 3	7	3	1.5
Site 4	19.3	4.5	2.5

NB: Measurements taken using an electronic trundle wheel. These measures are only approximate as a result of uneven terrain.

Appendix 7: Percent Cover Reference Guide

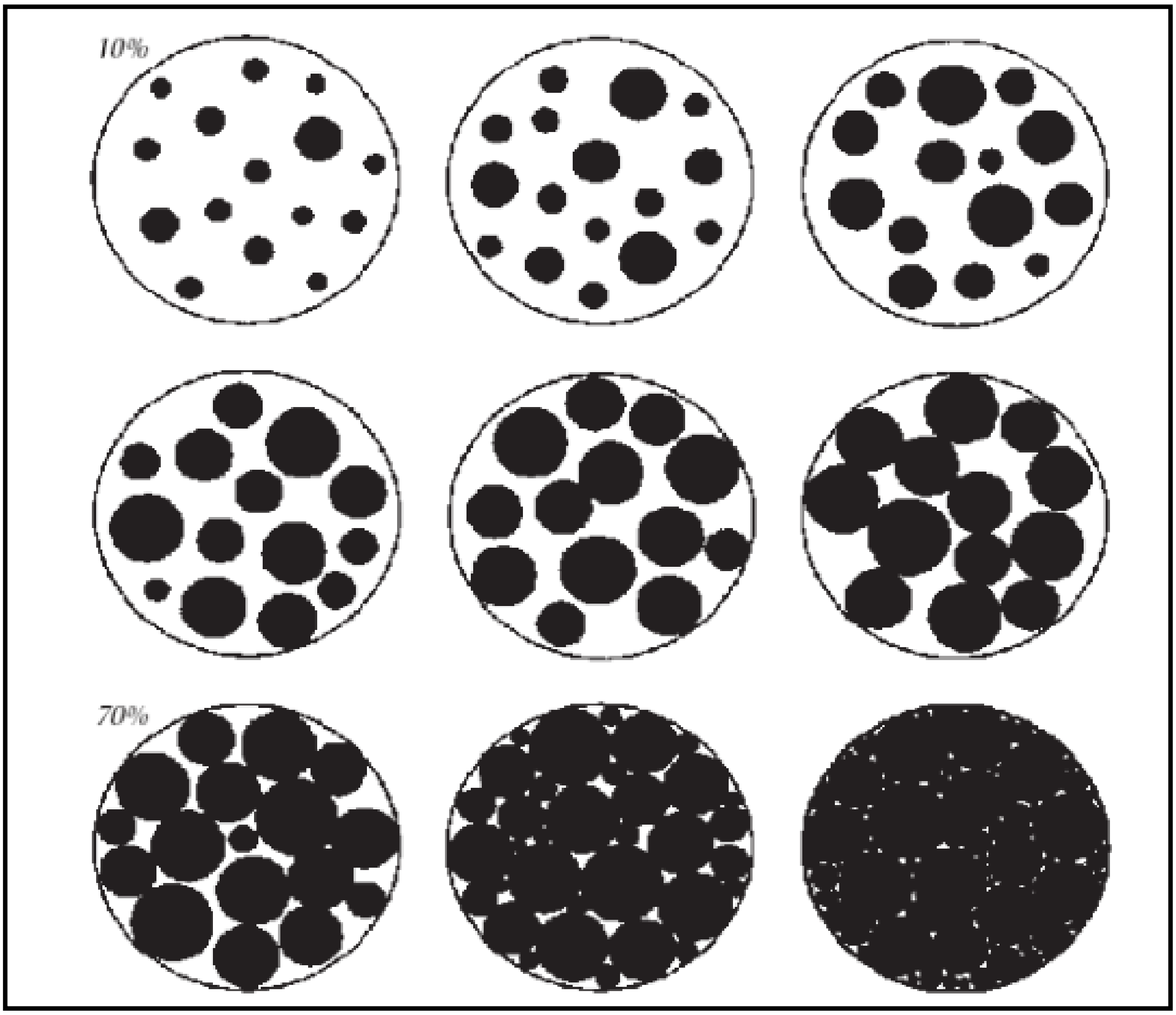

Figure 7a) Percent cover reference guide. Graphic representation of increasing levels of percent cover in a plot area. Starting in the top left corner with 10% cover and increasing in units of 10% from left to right, down to the middle left and to the right, then down to the bottom left to the right ending in 90% cover. Image taken from Center for Natural Resource Information Technology (CNRIT).

Appendix 8: Water Quality Plots for Sampling Locations

Forbes Creek

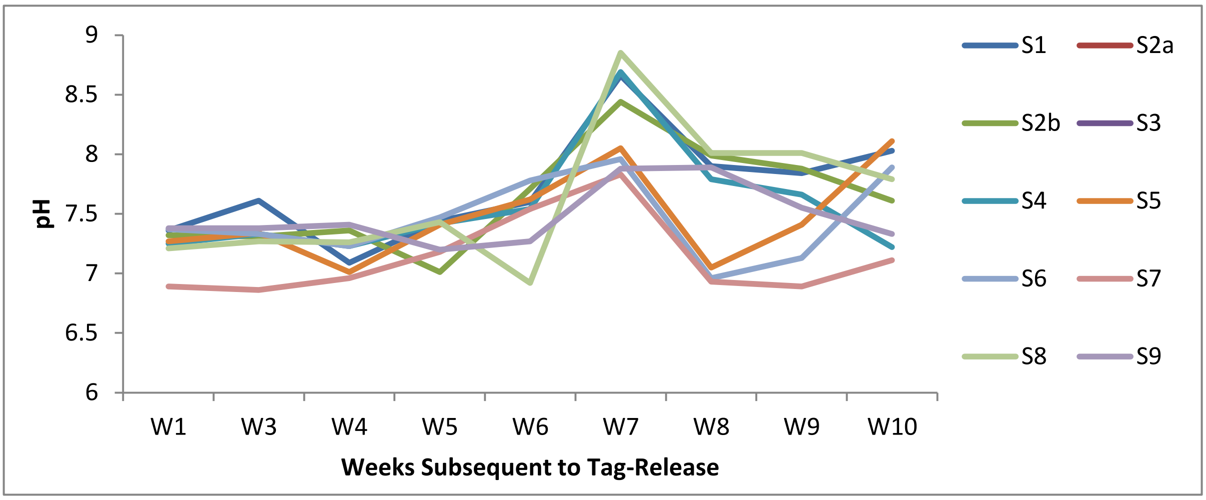

Figure 8a) Forbes Creek: pH levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

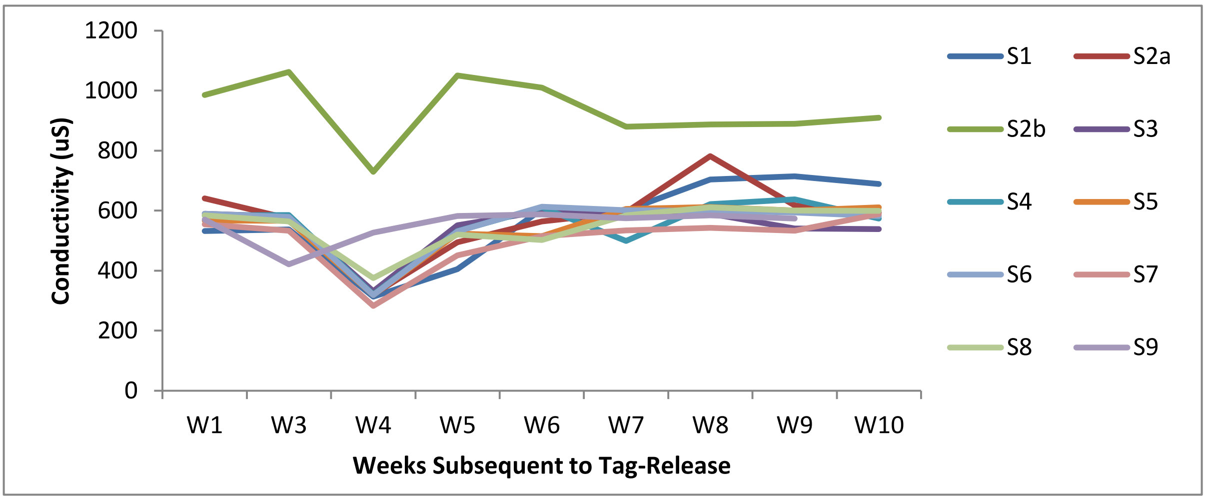

Figure 8b) Forbes Creek: conductivity (uS) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

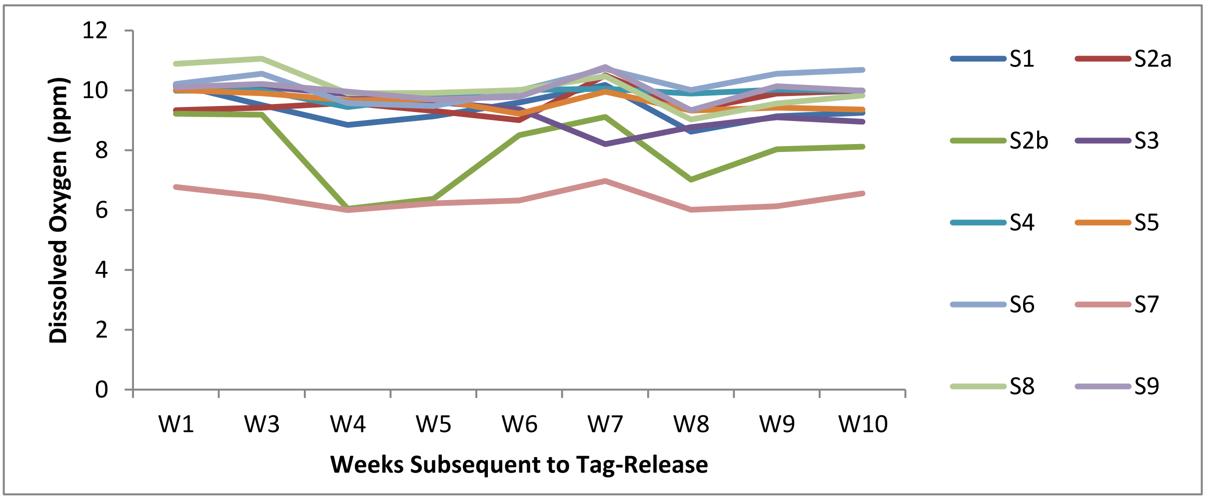

Figure 8c) Forbes Creek: Dissolved oxygen (ppm) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

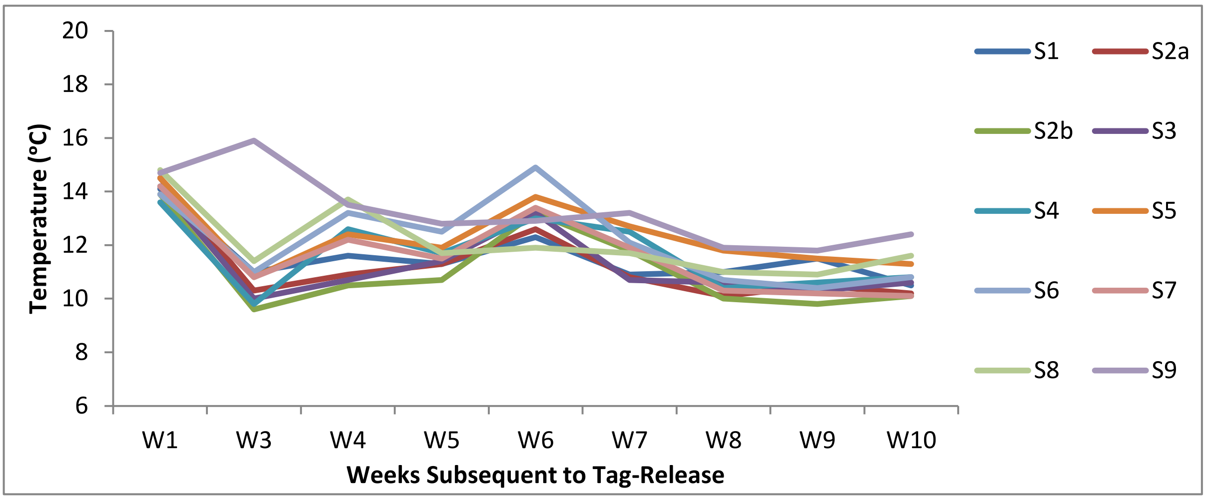

Figure 8d) Forbes Creek: Temperature (°C) at each sampling site throughout the experimental time frame (9 weeks).'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

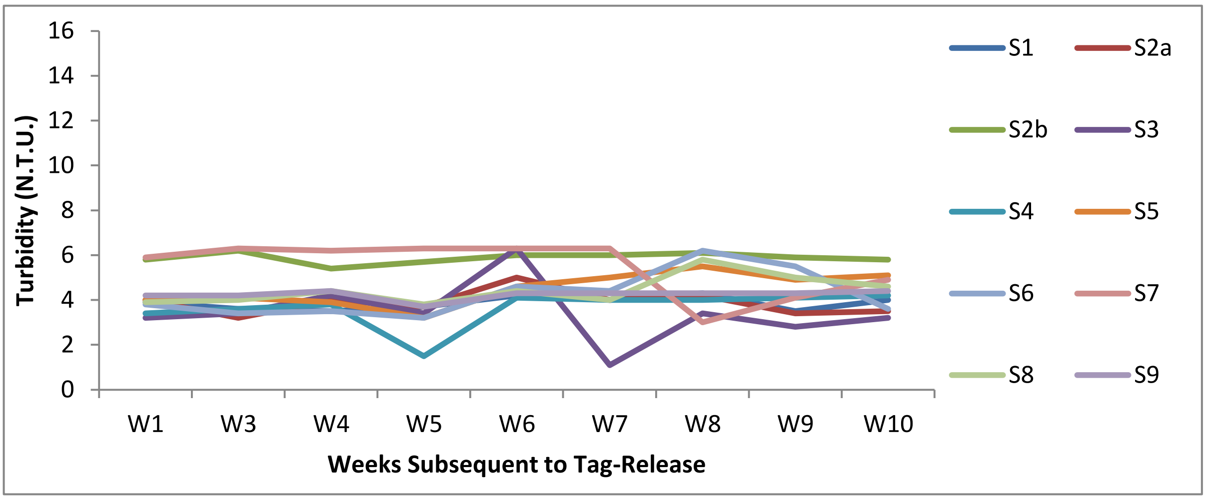

Figure 8e) Forbes Creek: Turbidity (NTU) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

Marton Park

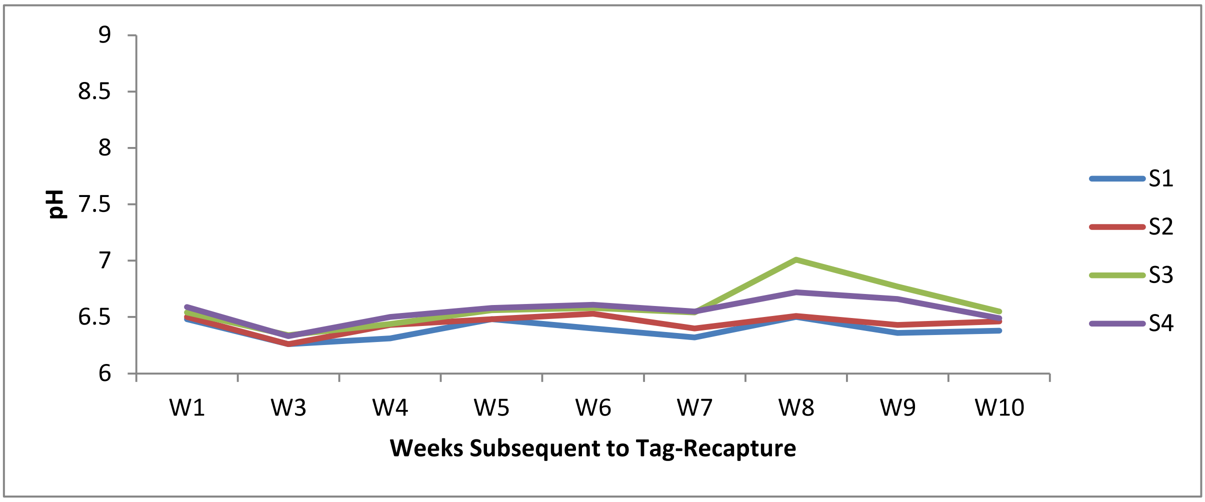

Figure 8f) Marton Park: pH levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

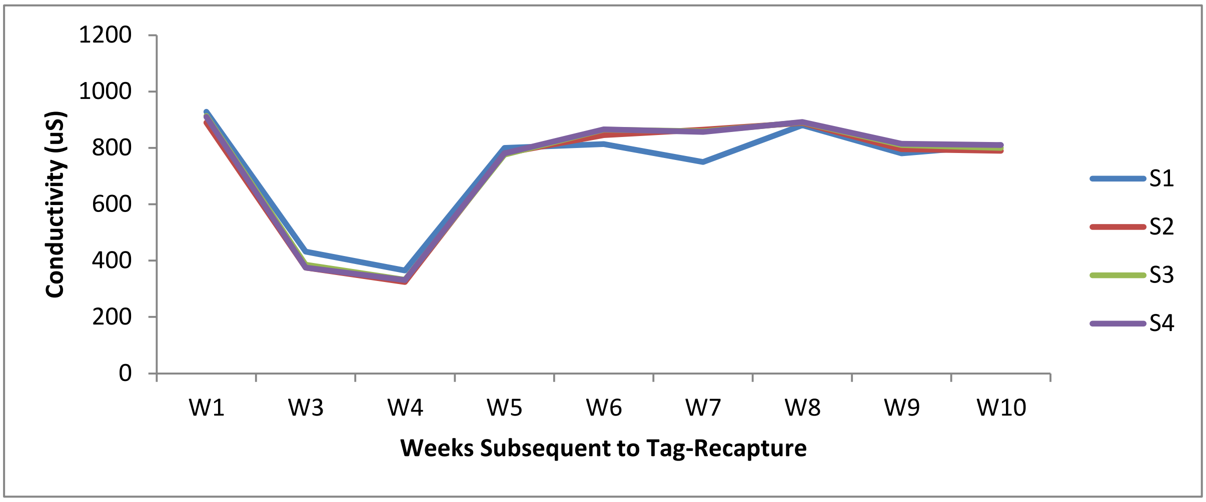

Figure 8g) Marton Park: Conductivity (uS) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

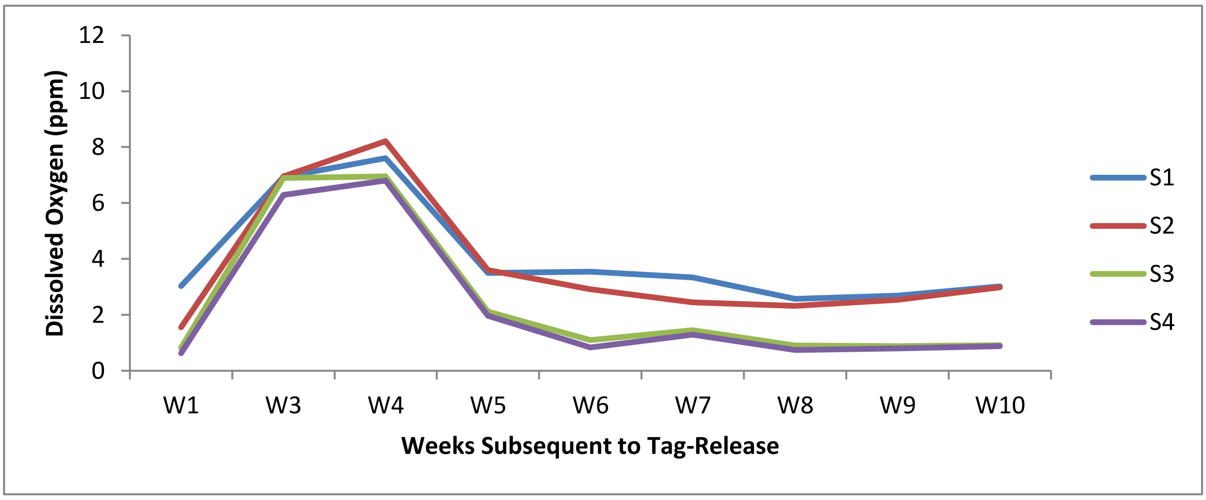

Figure 8h) Marton Park: Dissolved oxygen (ppm) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

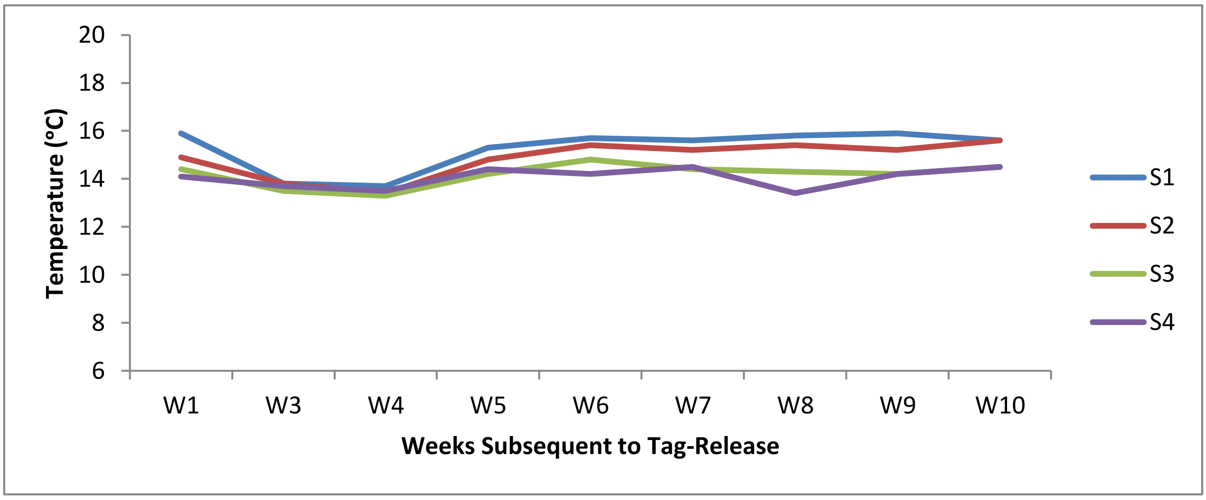

Figure 8i) Marton Park: Temperature (°C) at each sampling site throughout the experimental time frame (9 weeks).'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

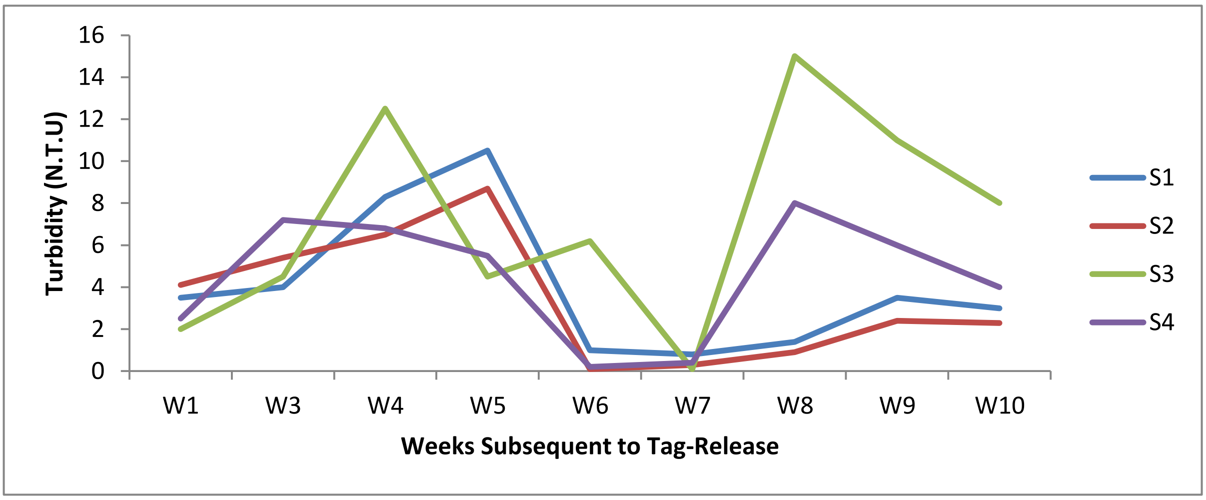

Figure 8j) Marton Park: Turbidity (NTU) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

Gwawley Creek

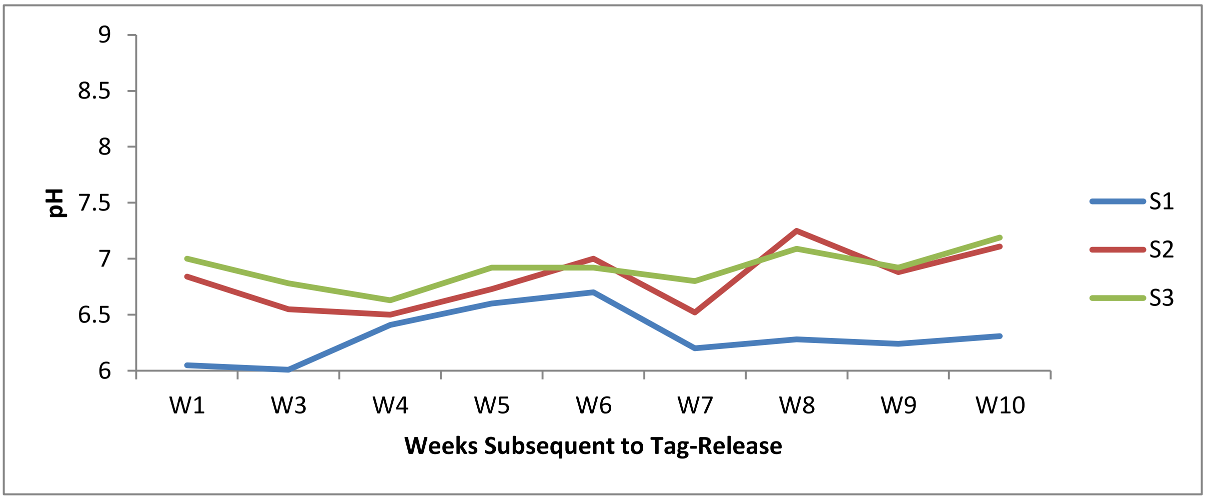

Figure 8k) Gwawley Creek: pH levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

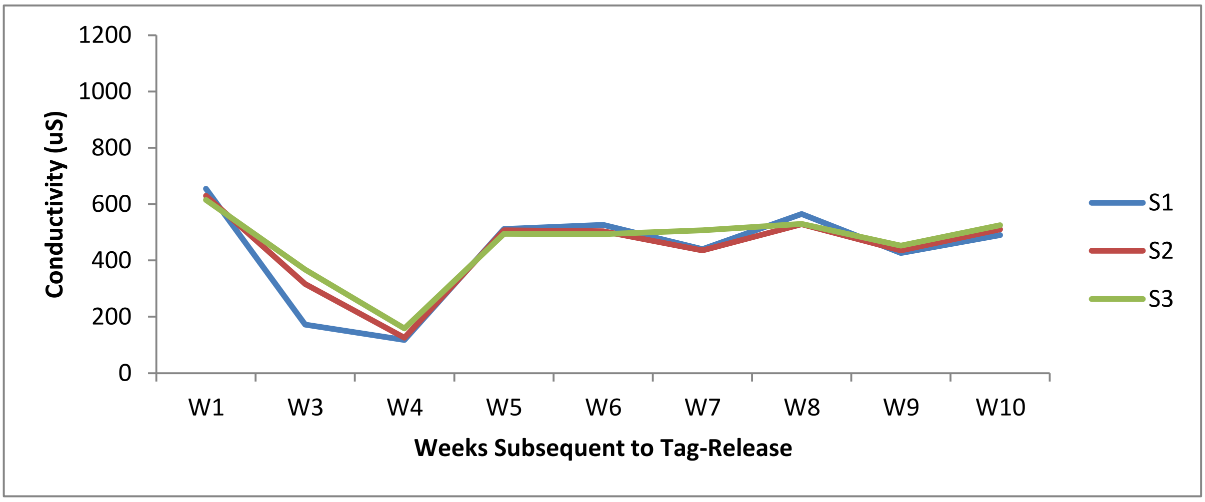

Figure 8l) Gwawley Creek: Conductivity (uS) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

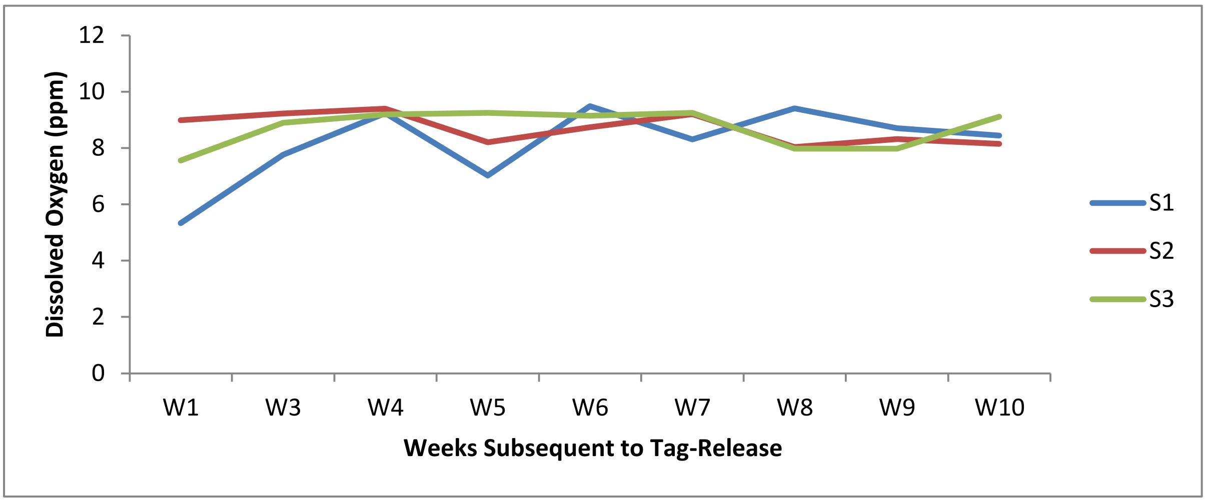

Figure 8m) Gwawley Creek: Dissolved oxygen (ppm) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

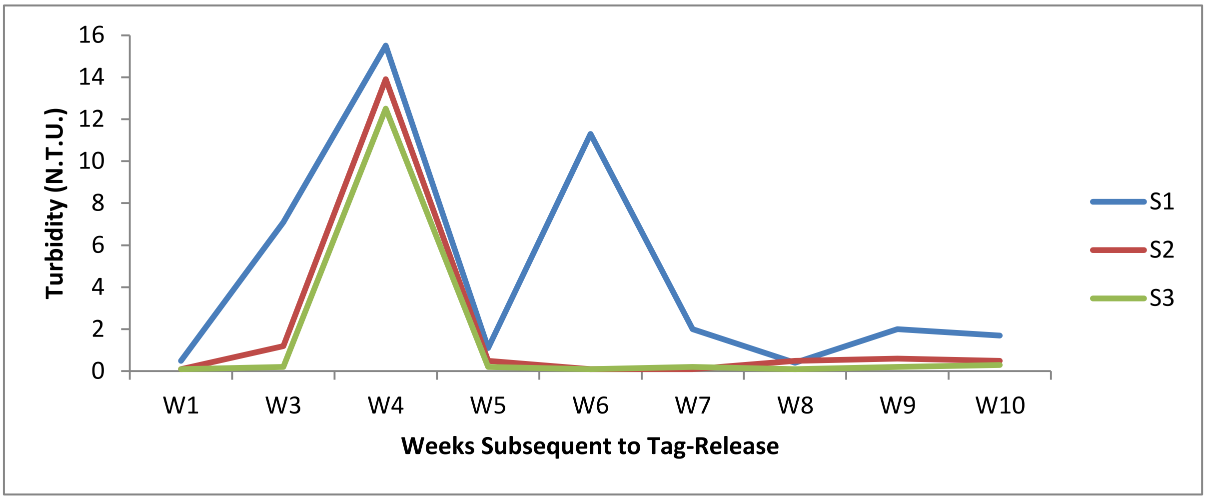

Figure 8n) Gwawley Creek: Temperature (°C) at each sampling site throughout the experimental time frame (9 weeks).'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

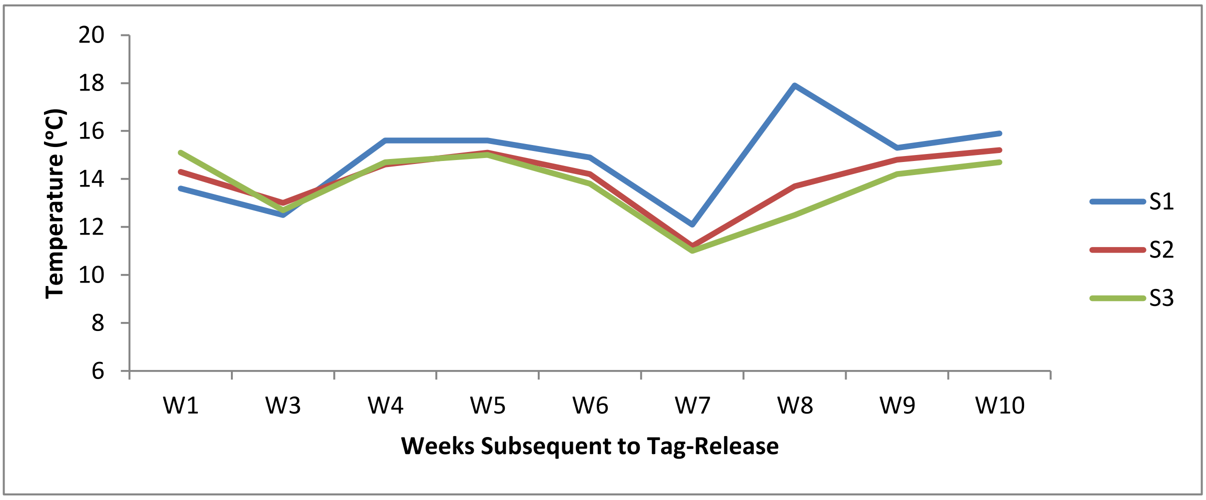

Figure 8o) Gwawley Creek: Turbidity (NTU) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

Carina Creek

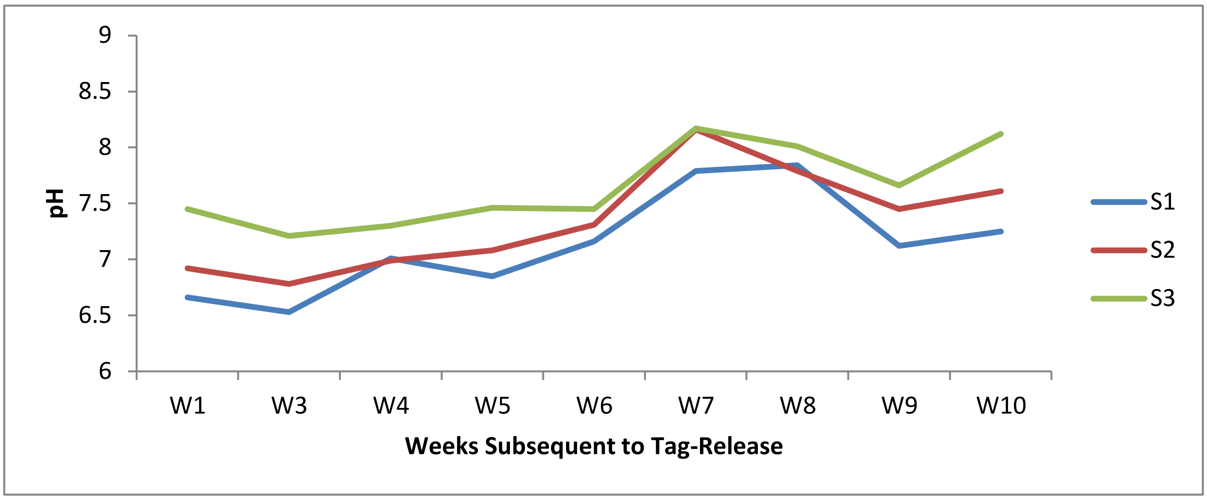

Figure 8p) Carina Creek: pH levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

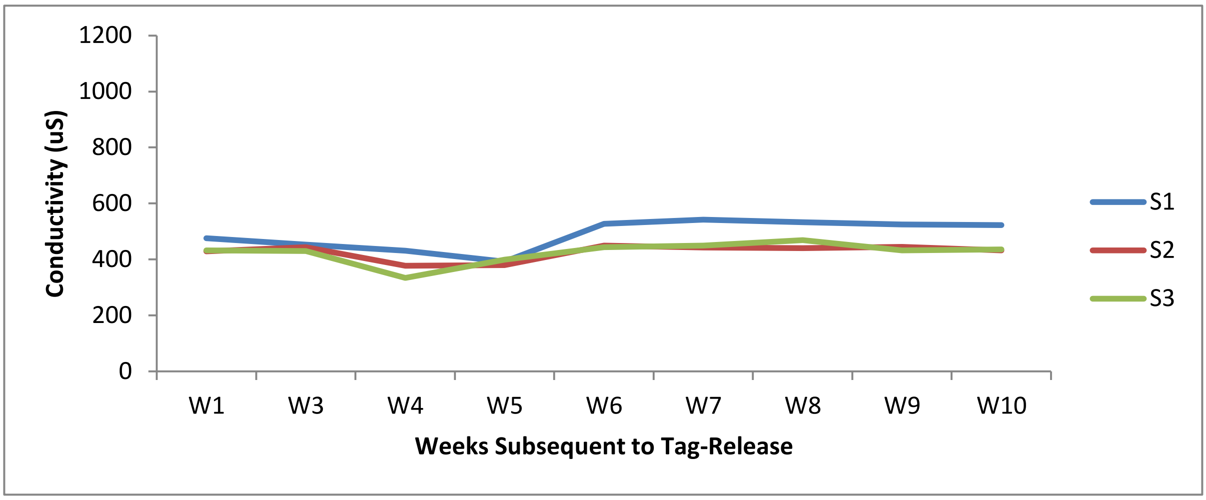

Figure 8q) Carina Creek: Conductivity (uS) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

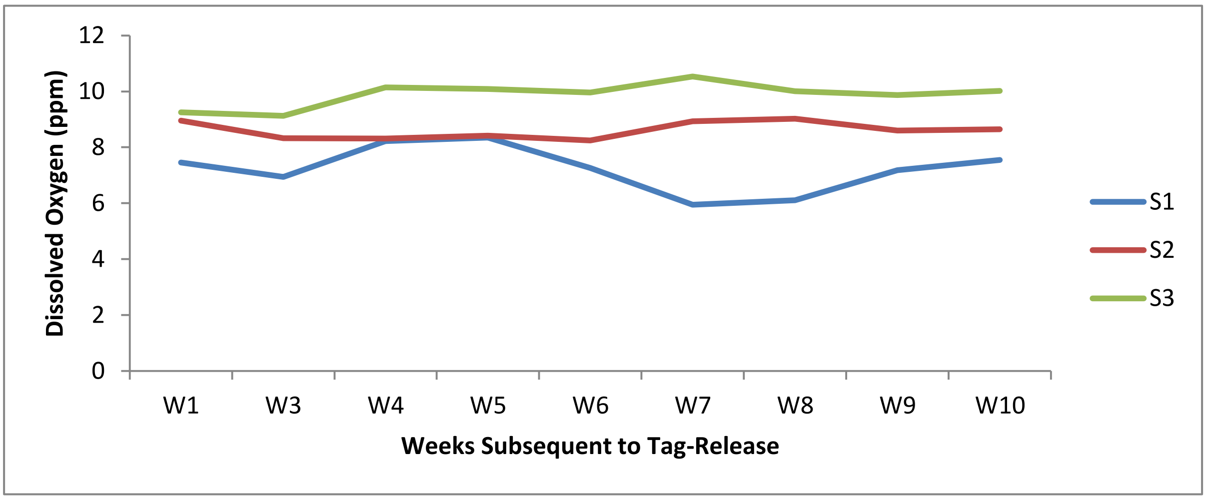

Figure 8r) Carina Creek: Dissolved oxygen (ppm) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

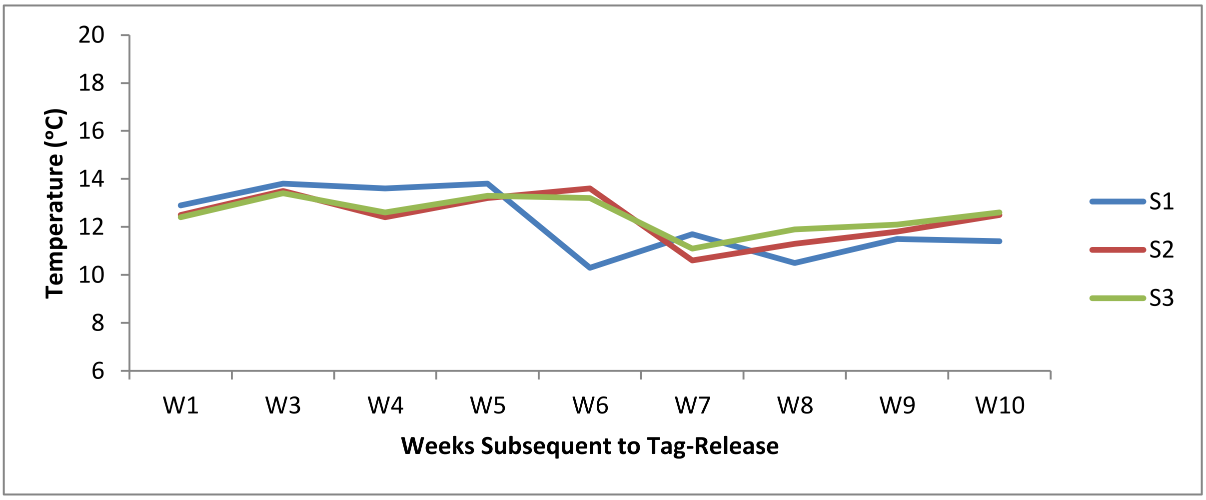

Figure 8s) Carina Creek: Temperature (°C) at each sampling site throughout the experimental time frame (9 weeks).'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

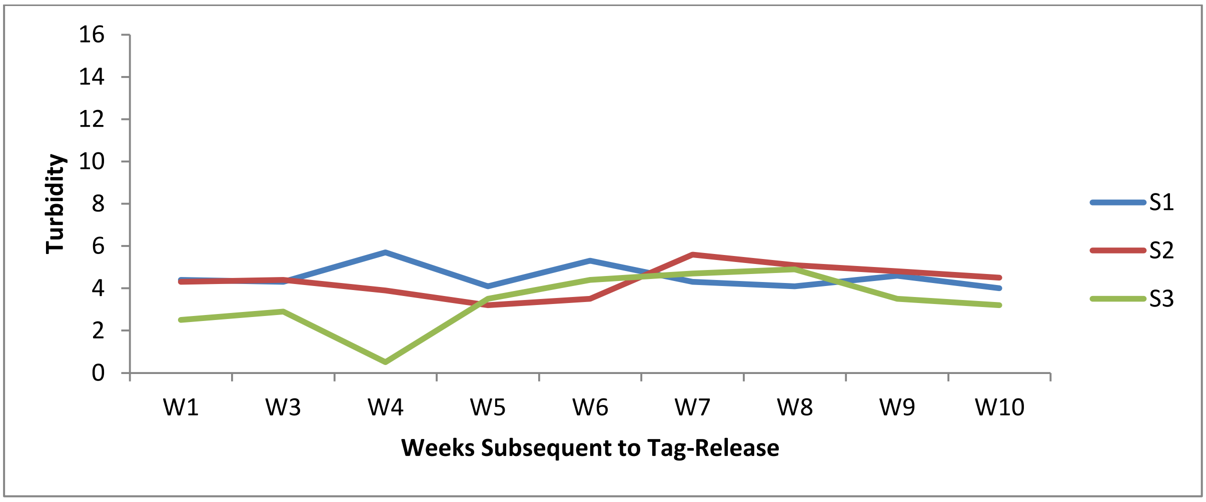

Figure 8t) Gwawley Creek: Turbidity (NTU) levels at each sampling site throughout the experimental time frame (9 weeks). 'S' denotes site; 'W' denotes week. Sampling was unable to be conducted in Week 2.

Appendix 9: Percent Vegetation Cover at Sampling Locations

Forbes Creek

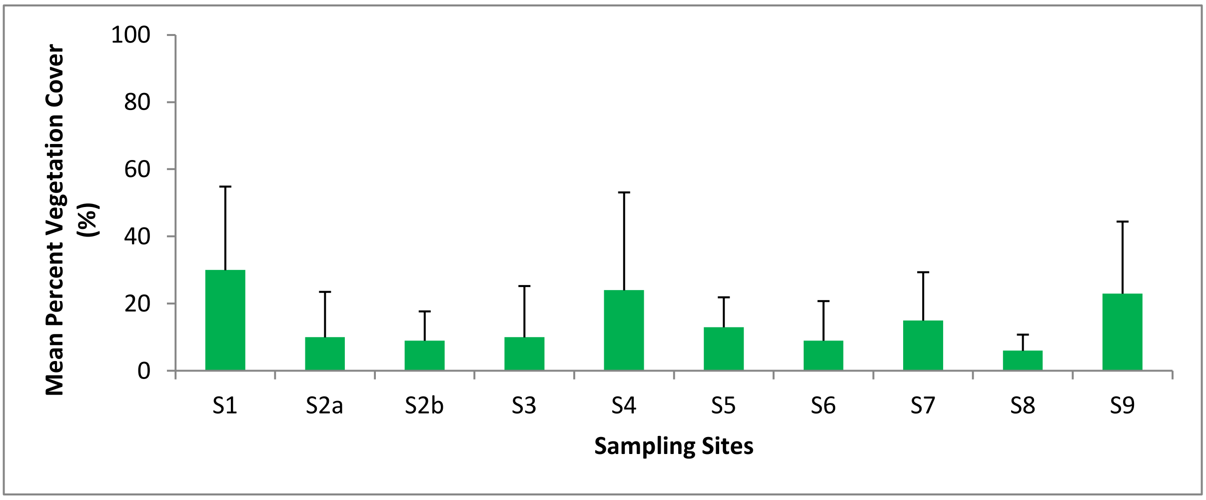

Figure 9a) Forbes Creek: Mean Percent Vegetation Cover (%) at each sampling site. 'S' denotes site. Error bars are representative of the standard deviation of quadrats analysed along each transect.

Marton Park Wetland

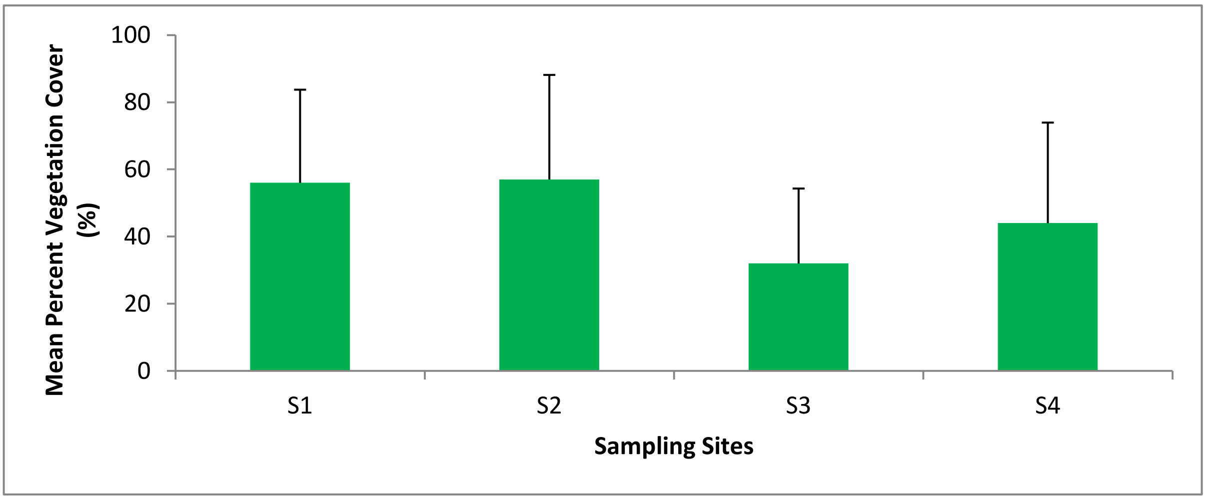

Figure 9b) Marton Park: Mean Percent Vegetation Cover (%) at each sampling site. 'S' denotes site. Error bars are representative of the standard deviation of quadrats analysed along each transect.

Gwawley Creek

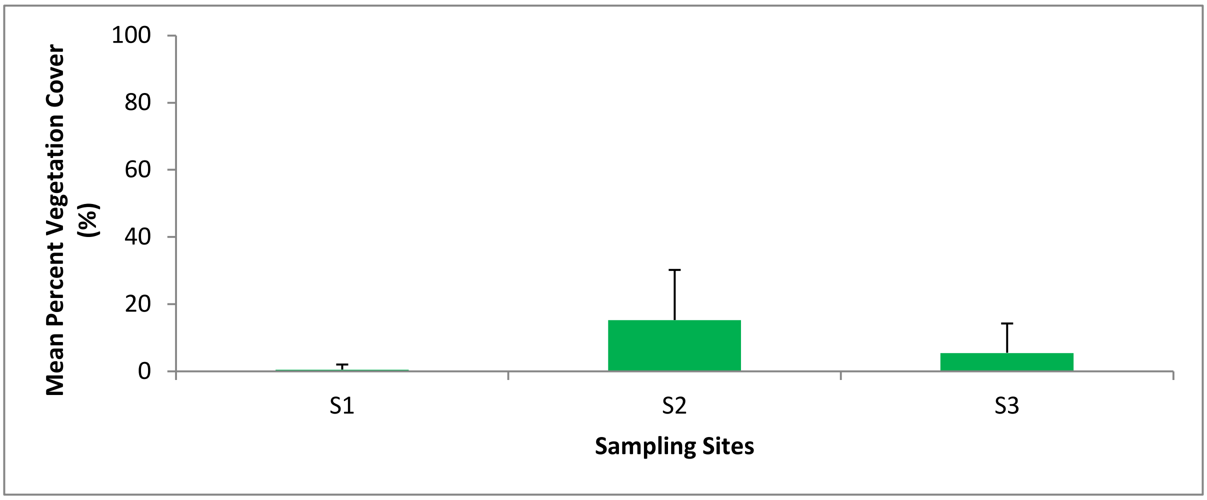

Figure 9c) Gwawley Creek: Mean Percent Vegetation Cover (%) at each sampling site. 'S' denotes site. Error bars are representative of the standard deviation of quadrats analysed along each transect.

Carina Creek

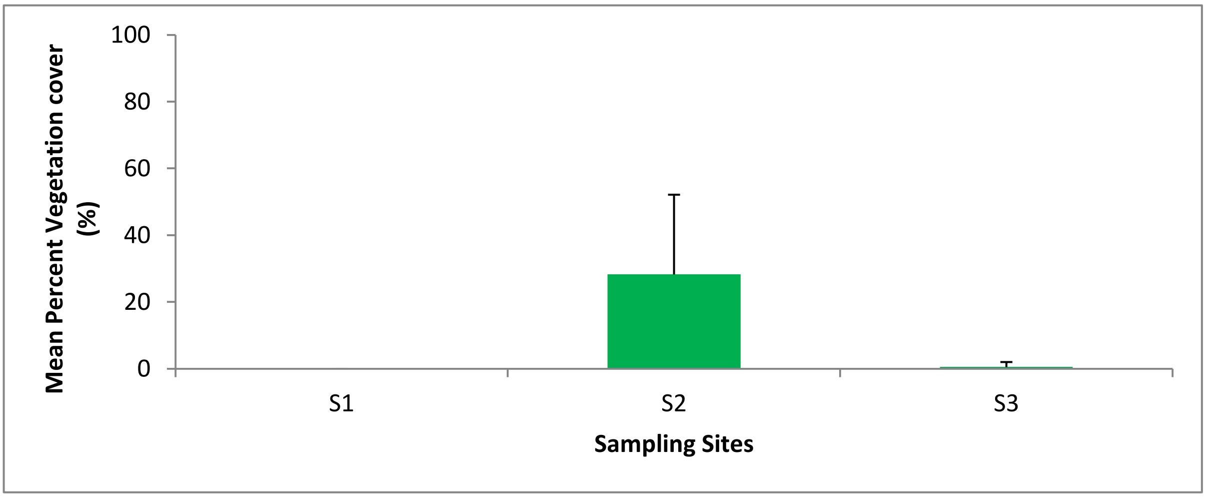

Figure 9d) Carina Creek: Mean Percent Vegetation Cover (%) at each sampling site. 'S' denotes site. Error bars are representative of the standard deviation of quadrats analysed along each transect.